The Senses of

FISH

Adaptations for the Reception of Natural Stimuli

Editors

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Foreword

FISH AND FOLK

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I love fish and when asked to write this *Foreword* was very happy to be given the opportunity to welcome this exciting tome on *"The Senses of Fish: Adaptations for the Reception of Natural Stimuli"*, edited by Profs. Gerhard von der Emde, Joachim Mogdans, and B.G. Kapoor. My enthusiasm was briefly damped when a graduate student of mine, whom I told of the task allotted to me, remarked "Who still reads books these days?". Temporarily stunned, I hastened to reply "Smart people do, of course!".

This volume contains such a wealth of information and presents such a wonderfully balanced variety of contributions by experts in their own fields, each and all expanding their subject one or another, that I could have added "This book makes smart people smarter still- and that's why smart people will read it". The topics covered fall neatly into six categories. The first two chapters, by Barbara Evans and Arnold Sillman & David Dahlin, provide the reader with exciting new details on fish eye adaptations and the reasons behind such adaptations. Eyes and vision in fish are finely tuned to the photic environment and as a consequence of this relationship as well as the wide range of habitats that fish occur in, a variety of modifications to the eye's optics, development, retinal organization, photopigments, absolute and spectral sensitivities have evolved. Without a thorough examination of these adaptations, we would be unable to make much sense of the visual behavior of fish.

Recent years have seen an upsurge of interest in the chemosensory abilities of fish and three chapters, one each by Anne Hansen & Klaus Reutter, Tine Valentinèiè, and Gabrielle Nevitt & Andrew Dittman, testify to this fact. Using behavioral responses and a comparative approach, Tine Valentinèiè shows that predatory fish with functional olfactory and taste receptors do not necessarily use these senses during food procurement. Visual hunters use the taste system solely during oral food evaluation. The situation for the detection of underwater smells is somewhat different as Hansen and Reutter demonstrate. However, that olfaction in migratory species plays a major role has been known for a long time, but how, for instance, imprinting in juvenile

salmon might work, in contrast to imprinting in mammalian species, is investigated by Nevitt and Dittman. The two authors provide evidence that odor memories may also be retained peripherally and that olfactory receptor neurons could be "selectively tuned" by responding to specific odors present "during a hormonally linked sensitive period". This is an exciting concept and one can expect more fascinating discoveries in this fertile field of 'developmental neuro-etho-ecology'.

A fascinating overview of the sensory systems and sensory brain areas in deep sea fish by Hans Joachim Wagner allows the reader not only to appreciate the specializations that are present in the denizens of deep water, but also to see the developmental parallels in fishes of other habitats (cf., for instance, Meyer-Rochow & Coddington, 2003, In: Fish Adaptations, eds. Val A.L. & Kapoor B.G., Oxford and IBH Publ. M/s Sci. Publ., Enfied, New Hampshire, pp. 339-383). This chapter by Wagner, placed in the centre of the book, thus connects the more detailed treatments of selected senses and sensory adaptations, preceding and following Wagner's chapter, with each other and, like the fish's brain, has an integrative function.

Aspects of sound production and hearing in fish are dealt with in another fascinating section of this book. Far from being silent creatures, Friedrich Ladich introduces us to the 'noisy' underwater world and describes how and why fish produce sounds. Little known and poorly studies is the ability of some species of fish to detect sounds as high as 180 kHz. Adaptations responsible for this remarkable feat, in particular within the Clupeiformes, and possible ecological consequences of ultrasound detection are being reviewed by Dennis Higgs. But how the acoustic signals in the end are 'heard' and processed by the fish's nervous system is the topic of Zhongmin Lu's chapter. Concentrating on what has been discovered on the goldfish's sound detecting pathways, Lu also examines acoustic communication and directional hearing in the mormyrid *Pollimyrus adspersus*, the midshipman *Porichthys notatus*, and other species. Hong Young Yan then summarizes the current state of knowledge with regard to hearing in fishes and emphatically draws attention to the fact that despite considerable progress, many aspects of sound perception in fish are still poorly understood.

A very comprehensive treatment by John Janssen is given to the role of the lateral line, especially in species that inhabit dark environments or turbid waters. Yet, how the different species of fish integrate the signals coming from the lateral line with those being received through channels of the other senses is still largely unknown. Joachim Mogdans, Sophia Kröther, & Jacob Engelmann in their chapter touch upon this problem, but deal primarily with recent findings on the lateral line brainstem's responses to a variety of stimuli. Two subsystems, consisting of superficial neuromasts whose function is impaired in running water, and canal neuromasts, whose function remains unaffected in running water, were discovered and led the researchers to conclude the presence of a clear form-function relationship for the lateral line.

Finally, the enigmatic electric sense of some fish: That topic forms the subject of chapters by Lon Wilkens, Tim Tricas & Joseph Sisneros,Clifford Keller, and Mary Hagedorn. Wilkens introduces us to the paddlefish, a fish that rarely features as an experimental subject. However, as Wilkens ever so ably demonstrated with his remarkable research, the paddlefish uses rostrally located electroreceptors to detect plankton and to investigate its environment. A form of

"passive electroreception", and what exactly this term entails, is revealed in the chapter by Tricas and Sisneros. The two researchers have chosen skates as their experimental subjects, marine species in other words, which, unlike the weakly electric fish (dealt with in the chapter by Keller), are not at all famous for communicating electrically. Keller leads us into an alien world of senses, into a world in which signals familiar to us humans have become largely replaced by electrical stimuli. Aimed primarily at conspecifics, such signals, like Mary Hagedorn explains, can also be a powerful incentive to those adventurous human beings, wishing to work with fish in remote jungle settings.

Although the contributors to this book hail from several different countries and presumably were not brought up in the same cultural environments, their fascination for fish unites them all. As someone who was born under the sign of *Pisces*, I too, have a "soft spot" for fish of all kinds: I like them fried, cooked, and smoked, and I regularly enjoyed 'gefilte fish' as a boy in my grandmother's home. But I am equally fond of them when I find them cut open in the dissection dish, under the microscope, or placed in some apparatus designed to reveal their physiological secrets. I love watching them dart around in my aquarium at home and have gone diving to see them in their natural habitats. I even have a collection of coins that feature fish and I know people, who possess thousands of fish stamps from all over the world. Obviously, as the enthusiasm has shown, with which this book's contributors have written their chapters, there are many different ways to express one's adoration for fish.

What makes fish so attractive to humans? Where does this age-old fascination for the slippery creature of the aquatic domain come from? Is it because we all pass through fish-like stages in our embryological development? Is it because the class Pisces outnumbers all other vertebrate classes with regard to the number of species (at least 25,000 species have been described to date)? Or is it, because fish are seen as inhabitants and messengers of a world alien, and maybe even threatening, to humans? I guess there isn't a single reason why humans since ancient times have found fish captivating, but fact is that the humble fish, as a symbol, has had a very long history and occurs in cultures and religions throughout the world. So fond must the early English have been of fish (or were they simply bad observers?) that they even lent this term to animals, which have little to do with real fish (witness shellfish, starfish, crawfish, jellyfish, cuttlefish, crayfish, and silverfish).

Christians revere the fish as a symbol of Christ and in Jewish lore the fish, being customarily consumed on the Sabbath, symbolizes prosperity. Pre-Christian Germanic tribes ate fish in honor of the great mother goddess Freya, a habit that according to some scholars persists to this day in the tradition of many a European to have a meal of fish on Fridays. In India a reincarnation of the goddess Durga, known as Meenakshi, was respectfully called 'the beautiful fish-eyed one' and the first Indian coinage of 600-300 BC has many examples of fish motifs. Several other non-Indian deities like Isis, Apollo, and Poseidon (the God of the Sea) were also associated with fish. Amongst the Maya of the New World different species of fish held different meanings: hags were seen as spies and destroyers, catfish represented cleaners and renewers, and other species were invoked in local systems of counting. In Japan, on the day known as 'kodomo no hi' (May 5th each year), even today tall bamboo poles with carp kites and streamers are erected in the

gardens of many a home, for to the Japanese the carp embodies strength and determination. On the other hand, fear and panic have nowadays, unfortunately, become almost synonymous with sharks around the world. However, actual shark-worshippers still inhabit some remote islands like Malaita of the Solomons in the West Pacific.

Clearly, fish have accompanied humanity and human culture from their beginnings, and this has also found numerous reflections in the arts. Poems in praise of fish have been written and set to music (F. Schubert's composition "Die Forelle" comes to mind), fish paintings (e.g., Paul Klee's "Golden Fish"), fish sculptures (the Olympic fish of Barcelona beating them all with its gigantic size), and nowadays even fish movies, fishing competitions, and fish games exist. Stories, fairy tales, and fables involving fish, often rooted in antiquity (who would not have heard of Aesop's 'Fisherman and the Little Fish'?) have a long history, but they are still popular today and they are being told in virtually all parts of the world.

This book, although anything else but a collection of mere fishy stories and fables, does follow that long tradition of human fascination with fish and there is not doubt that the scientific research, reported in this volume, represents the fruits of years of patient and dedicated study. The authors, editors, and publishers must feel privileged to share in the production of so worthy a text as I do to have been called to welcome it on its appearance. As we gather more and more data on the wondrous ways the fishes 'function', we slowly start to grasp why, for at least 400 million years, these animals have managed to flourish and been able to live through geo-historic crises and environmental changes too profound for us to comprehend. We are reminded of the fact that almost three quarter of the Earth's surface are the domain of our friends, the fish. And we are realizing that we are in the process of understanding, what kinds of adaptations have allowed them to succeed in occupying every conceivable niche of their watery realm and what roles their sensory capacities have played to guarantee their survival through the ages.

Much of what we know today about the senses of fish, represents abilities and adaptations almost unimaginable to us 'mere humans' only a few years ago. This is a fact and there is nothing 'fishy about that. However, none of the exciting discoveries can diminish the deep respect we hold for our 'scientific quarry'. On the contrary, the discoveries have served to enhance our appreciation of this formidable creature of Nature and sometimes may even cause us to take a deep breath and reflect on our own imperfections.

Before I end this Foreword with a translation of that fitting and famous poem "Die Forelle/ The Trout" by Christian Schubart, I should like to emphatically state: This book cannot fail to give great pleasure to all those people smart enough to pick it up and read it thoroughly!

The Trout

Beneath the limpid water
I saw a jolly trout
As swift as arrow darting
And flashing in and out.

I stood upon the margin,
All in the morning cool,
And watch'd the fish disporting
Down in the crystal pool.

Near by there stood an angler,
With rod and line and hook,
And saw the trout a-swimming
Down in the crystal brook.

With shine upon the water
Unbroken, so I thought,
In vain will be his angling,
The fish will not be caught.

At last the thievish angler
Impatient grew. With guile
The water clear he ruffled,
And in a little while

He jerk'd his rod in triumph.
And struggle, struggle as it may,
The poor outwitted victim
Upon the greensward lay.

From: Garran R.R. 1946 (reprinted 1971) "Schubert and Schumann". Melbourne University Press

Preface

Animals have to be able to perceive external sensory stimuli and generate appropriate behavioral responses in order to live successfully in their environments. On an ontogenetic scale, behavioral responses to sensory stimuli are either innate or learned. Learning enables an animal to respond to changing stimuli through experiences made during life. On a phylogenetic scale, organisms also 'learn' to adapt to changed signals in their environment. Evolutionary learning works in a different time frame, however, that is from generation to generation. Its motor is selection, and its result is an adaptation of the sensory apparatus and the nervous system. During this adaptation process, 'communication' with the environment is maintained, enabling the animal to gather important signals coming either from the surroundings or from other animals, like prey, predators, or conspecifics.

In this book we use fish as model systems to demonstrate how evolutionary learning has shaped various sensory systems. Why fish? There are three main reasons for this: First, 'fishs' comprise a huge and diverse group of animals. There are about 25000 extant fish species that live in such diverse environments like the deep sea or shallow freshwater puddles in the Himalayas. Closely related taxa may live in different habitats and thus under completely different environmental conditions. They have adapted to these diverse environmental conditions not only in their external body features, but also by modifying their sensory apparatus. Second, fish possess more senses they many other animals. They are equipped with the well known senses that humans have, but in addition they possess at least three more senses. Third, fish stand at the basis of vertebrate evolution. While shaping the senses of fish, evolution worked on the very 'blueprint' of vertebrate organisation, which forms the basis of all other vertebrates, including humans. Fish thus can serve as model systems for vertebrate evolution in general, including the evolution of sensory systems and their adaptations to environmental conditions.

We brought together scientific experts from different areas, who report about the sensory systems of fish and unravel their physiological and ecological evolutionary adaptations. The first two chapters deal with vision, one of the most important senses of vertebrates. However, vision in different aquatic habitats has to cope with quite diverse photic conditions and thus requires fundamental adaptations of the eyes and their sensory receptors. Chemoreception, dealt with in chapters three to five, may be one of the "oldest" senses of vertebrates. Yet again,

environmental conditions differ in the various habitats fish live in, and this requires adaptations of the olfactory and taste systems of the different groups. An interesting case study of olfactory specialization is olfactory imprinting, which can be found in several groups of fishes. The best-known example may be olfactory imprinting in the service of homing in salmons as described in chapter five. Chapter six does not deal with a particular sensory system, but takes a look at several groups of fish living in an extreme environment: the deep sea. Here, the chemical senses, vision, and the lateral line system are especially developed and adapted to the different niches in this ultimate environment. That fish are not deaf is well known since the days of Karl von Frisch, and chapters seven to ten discuss several aspects of underwater hearing. Related to hearing, sound production by fish (chapter 10), mainly in the service of intra-specific communication, is an important evolutionary factor shaping fish hearing organs. The lateral line system of fish compromises two main sub-systems: the mechanosensory lateral line, dealt with in chapters 11 and 12, and the electrosensory lateral line (chapters 13 to 16). The latter can be divided still further, depending on the type of stimuli that are perceived: low frequency electric signals for 'passive' electrolocation through ampullary electroreceptor organs (chapters 13 and 14), and high frequency signals, actively produced and thus involved in 'active' electrolocation (chapter 15). Finally, chapter 16 stresses an important 'human' point: science is made by people, sometimes under extreme conditions. Especially when studying environmental adaptations of animals, it is necessary to go out into the field and get information directly from the source. This can be dangerous at times and may require personal sacrifices by the scientists that are often forgotten and not mentioned in scientific reports, thus making scientific results look simple and 'easy' to obtain. That this is often not the case is highlighted by our final essay-chapter.

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A Fish's Eye View of Habitat Change

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ABSTRACT

In aquatic ecosystems, visually mediated predator-prey interactions are highly dependent on the environmental light regime; however, the wavelengths of ambient light and thresholds of light intensity vary as a function of water depth and dissolved organic matter. Light is also scattered by water molecules creating polarized light and by silts and clays creating turbid conditions. As a result of these changes in the visual environment, the visual systems of fishes have developed many adaptations, and are finely tuned to the spectrum and intensity of light in the microhabitat.

In addition to these physical constraints, natural selection favors prey that are harder to detect by the predator. To improve the probability of detecting these cryptic prey, many planktivorous fish utilize "saltatory search", a behavior which is intermediate between ambush and cruise search strategies. Foragers using a saltatory search strategy only search during stationary pauses between repositioning movements. To further improve their foraging success, these fish systematically change the components of search in response to changes in the prey assemblage and light environment.

Variation in environmental light is a selective pressure for changes in foraging behavior, but also leads to changes in the fish eye. The structure of the eye, retinal organization and visual pigments of fish all vary as a function of ambient light conditions. These visual system variations can be classed as 1) reversible, where the fish respond to changes on a daily, seasonal or migratory cycle; or 2) irreversible, where the fish undergo a transformation or metamorphosis in preparation for life in a new habitat. The life history strategy and the light environment of the visual habitat can explain variable timetables of retinal development.

The visual habitat of fish is a complex niche varying in the quality and intensity of light. The corresponding diversity revealed in the structure of the visual system in fish is dynamic and intriguing. The visual ecology of fish is directly affected by the environmental light conditions. Vision is very important to foraging success and predator avoidance in fish, which are important selective pressures in the adaptation of the retina/fish eye to visual habitat. Understanding retinal structure and function in the context of behavioral ecology is an area rich for further research.

Key words: Light, Retinal development, Metamorphosis, Cone mosaic, Saltatory search

INTRODUCTION

The diversity of both sensory structures and visually mediated behaviors of fish reflects the complexity of their environment. In aquatic ecosystems, visual stimuli vary in both physical and

biological components. The physical light environment can vary in wavelength (color), intensity (brightness), and scatter (turbidity and polarization); and many microhabitats differ in these qualities of natural light. Biological components include both prey and predator stimuli and visual cues are commonly used to guide foraging for prey, vigilance for predators and preda-tor evasion. Changes in the density and species composition of predator and prey add to the complexity of the visual ecology of fishes. Light intensity and wavelength at depth change daily, and fish often change habitats throughout their life history. Such continual environmental change is a major selective pressure for the adaptive radiation of visual systems in fish.

In response to environmental change, fish exhibit a wide variety of developmental strategies. Fish have indeterminate growth, and even the eyes continue to grow throughout life. Such developmental plasticity enables fish to modify sensory structures with changes in habi-tat, allowing natural selection to act at various times during the life history. In this review, some general principles of visually mediated search behavior and retinal development are presented. The range of natural stimuli in the environment and the spectrum of evolutionary adaptations in fish are discussed, as well as how individual fish cope with changes in environmental light and prey characteristics.

NATURAL STIMULI

Intensity and Wavelength of Light

When viewed from above the water surface, aquatic habitats appear as uniform visual environ-ments, but beneath the surface they are visually dynamic. The intensity of light decreases exponentially with depth as a result of absorption and refraction of light, and is referred to as vertical light attenuation (reviewed in Lampert and Sommer 1997). In addition, different colors of light are not equally absorbed by water. Long wavelengths of light (reds & infrared) are absorbed in the upper meters, whereas shorter wavelengths (blues & ultraviolet) penetrate deeper into the water column. Thus, both the intensity and spectrum of light change from the surface to deep water.

In addition to being absorbed by water molecules, light is highly absorbed by organic matter dissolved in the water (Jerlov 1968). Pure water transmits ultraviolet light (UV) and appears blue. Vegetative decay adds yellow compounds such that water appears green. With greater quantities of dissolved organic material, the water may appear "tea" colored and transmits only longer wavelengths of light (red) and in some cases only infra-red, such as in "blackwater" (Levine and MacNichol 1982). Increasing levels of organic matter are encountered as one travels from open ocean to coastal ocean, and on into freshwater environments. As a result, light in the open ocean is characteristic of pure water, whereas coastal ocean and freshwater fish habitats have a light regime shifted to longer wavelengths (Levine and MacNichol 1979; Lythgoe 1984).

Sunlight is the only light source in freshwater habitats, but in the ocean there is also biolu-minescence (Douglas et al. 1998). Downwelling sunlight is the main light source from the surface down to 1000 m in clear, ocean water. At this depth, the ambient spectrum is restricted

to blue light (470-480 nm). Species present between 200 and 1000 m utilize both downwelling and bioluminescent light cues (Partridge et al. 1988), but below 1000 m, bioluminescence is the only source of light, and is usually present at wavelengths of 450-500 nm (Marshall 1971). Although bioluminescence is rare in freshwater fish species, 80% of deep-sea species can produce light, most likely employed as camouflage against the background of downwelling light (Anctil 1979).

Scattering of Light

Light can be scattered both by water molecules (creating polarized light) and by suspended silts and clays (creating turbidity). Both types of scattered light impact the visual ecology of fish.

Polarization

Horizontally polarized light, scattered by water molecules, may provide orientation cues to aquatic animals (Waterman and Forward 1972; Schwind 1999; Wehner 2001), and may also enhance detection of zooplankton prey by fish. Although zooplankton are transparent, their birefringent body surfaces reflect polarized light. When viewed with a polarization sensitive visual system zooplankton become more visible (Novales Flamarique and Browman 2001). Possibly this reflected polarized scatter is more prevalent in the UV spectrum. Shorter wavelengths of light, such as ultraviolet, are thought to be more highly scattered (Lythgoe 1979). However, the measured degree of polarization in coral reef environments does not differ over the spectrum from 360-550 nm (Cronin and Shashar 2001).

Turbidity

Suspended silt and clay scatters light, and greatly reduces the vertical penetration of light into the water column (Grinstead 1965). Although turbidity is often equated with degraded ecosystems, some ecological communities continue to thrive under these conditions (Schulz et al. 1999). Similar to low light conditions, turbid conditions provide refuge to prey from visual predators. For example, in turbid conditions, the susceptibility of zooplankton to encounter by planktivorous fish is reduced as a result of the decrease in reaction distance (Vinyard and O'Brien 1976), and planktivores are protected from piscivores by the same mechanism (Vogel and Beauchamp 1999).

Prey and Predator Stimuli

Ecological implications of the natural light environment are manifested through the detectability of prey and predators, both of which are subject to the limitations of vision (Howick and O'Brien 1983; Dunbrack and Dill 1984). Light intensity, wavelength, and scattering of light all affect distances at which prey can be detected. (Vinyard and O'Brien 1976; Browman et al. 1994; Novales Flamarique and Browman 2001). Natural selection favors predators with a better chance of detecting prey (O'Brien 1979), but also favors prey with less chance of being detected.

A number of options are employed to evade and avoid visual predators. Evasion of predators is achieved by schooling behavior (Pitcher 1980; Pitcher and Turner 1986). Schooling prey do not avoid detection, but use the school to evade capture. Avoidance of predators can be achieved through camouflage using bioluminescence (Case et al. 1977), cryptic coloration (Fujimoto et al. 1991) or transparency (McFall-Ngai 1990). Another means of predator avoidance is vertical migration. Many fish descend deep into the water column by day and return to the surface at night. Some fish vertically migrate to avoid predators (Blaxter 1976; 1986), while others follow their prey into deeper water (O'Brien et al. 1984).

Given the many methods used by prey to avoid predation, foragers need an effective search behavior to increase the probability of detecting prey. Visual foragers have typically been classed as either cruise or ambush searchers, and classic ambush predators are common (e.g. Kunz et al. 1985). Ambush predators remain motionless until a prey comes into range, at which point the predator initiates attack (Curio 1975). Cruise searchers are presumed to move constantly, searching for prey while moving; however, few species actually utilize a true cruise search strategy (O'Brien et al. 1990). Instead, a more commonly used strategy is the saltatory search behavior described for planktivorous fish (Evans and O'Brien 1988). Species utilizing saltatory search do not search while moving (Fig. 1). Instead, search occurs during stationary pauses between locomotory "runs" (O'Brien et al. 1986; O'Brien and Evans 1991). Stopping to search allows greater efficiency in prey detection and fish using this strategy are able to alter their search behavior as a function of prey detectability.

THE FISH EYE: STRUCTURE AND FUNCTION

How the visual environment affects prey detectability ultimately depends on the anatomy and physiology of the fish eye. The vertebrate eye is very similar from fish to humans (Walls 1942),
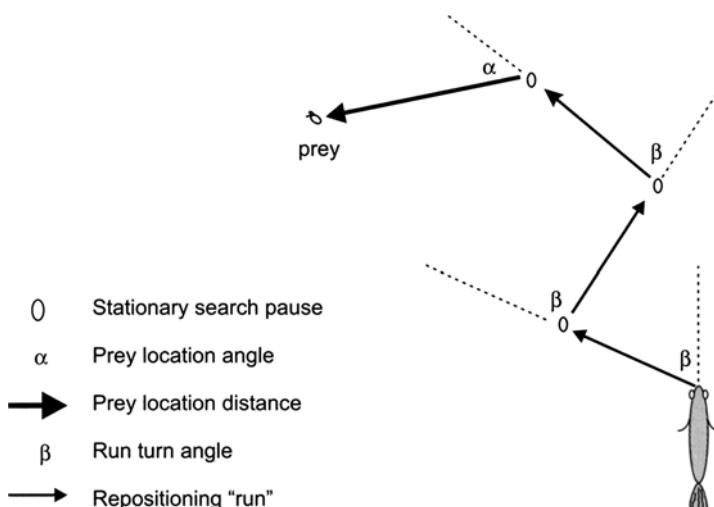

Fig. 1   Saltatory search. The components of the search path are illustrated for a forager using saltatory search. In this example, a plankton feeding fish moves intermittently, stopping at intervals to search. The time spent in search, the distance traveled, and the turn angles all vary with prey detectability.

and the main optical components are the cornea, lens, and retina (Fig. 2). In contrast to terrestrial animals where the cornea provides a significant amount of refraction, the fish cornea has the same refractive index as water and is optically absent. As a result the fish lens is typically spherical to provide a sufficient index of refraction to focus an image on the retina (Fernald 1988; Hawryshyn 1997). The types of photoreceptors present in the retina, the photopigments they contain, and their pattern of distribution throughout the retina, are determined by the specific light environment of the fish.

Retinas of Fish

Detection of light occurs in the retina. In vertebrates, the neural retina is part of the central nervous system (CNS) and uses the same class of neurotransmitters as other CNS neurons (Gordon and Bazan 1997). Light entering the eye passes through the cornea, lens and inner retina before reaching the photoreceptor cells (Fig. 2). The rod and cone photoreceptor cells transduce light information into coded neural impulses (Schnapf and Baylor 1987) which are then further processed by the horizontal, bipolar and amacrine cells. Ganglion cells relay this coded information through their axons to the brain (Wulliman 1997). Each ganglion cell has a receptive field of photoreceptor cells through which it "sees" the environment. The neural circuitry of the retina creates center-surround fields which increase visual contrast (Bilotta et al.
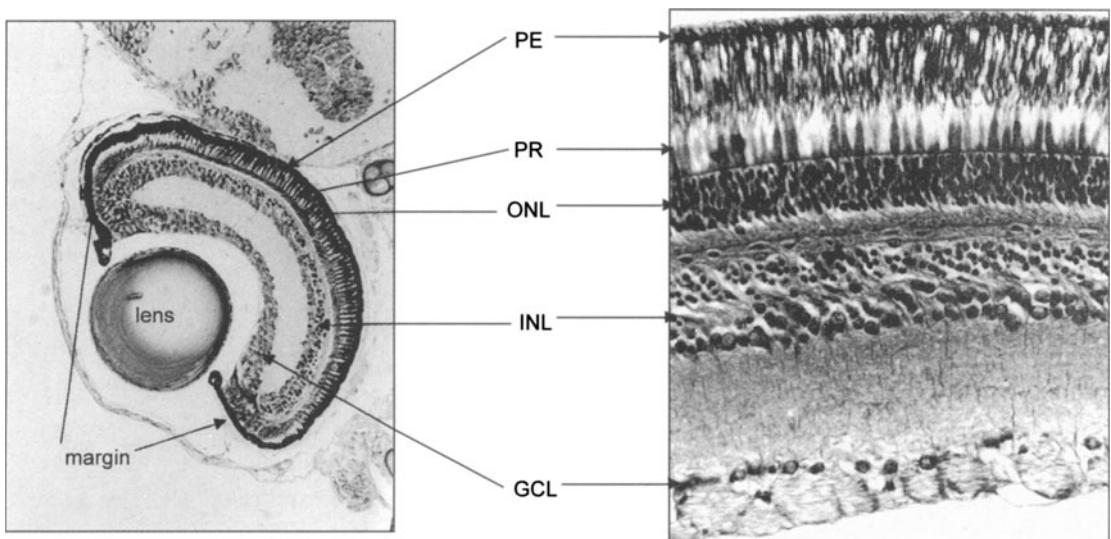
Fig. 2 Structure of the fish eye and retina. Cross sections (3 microns) of winter flounder eye. Left panel: pure cone larval eye (40 dph) illustrating the spherical lens and neural layers of the retina. Right panel: post-metamorphic retina (10 cm) illustrating rod and cone photoreceptors: PE: pigmented epithelium; PR: photoreceptors; ONL: outer nuclear layer (rod and cone nuclei, horizontal cells and rod progenitor cells which give rise to new rods); INL: inner nuclear layer, (bipolar and amacrine cells and a population of multipotent progenitor cells); GCL: ganglion cell layer (axons of ganglion cells form the optic nerve; not shown); margin: mitotic region producing all retinal cell types.

1995). Ganglion cells may be either excited, or inhibited by light in the center of their receptive fields, depending on whether they receive input from "on", or "off" center-surround fields, respectively (reviewed in Hawryshyn 1997). Electrical recordings from ganglion cells indicate that both on and off center-surround receptive fields are present in fish retina (Burkhardt 1977; Beaudet et al. 1993).

Photoreceptor Cells

The visual receptor neurons of the retina are the rod and cone cells, originally classified by their shape (Walls 1942). Both rod and cone photoreceptors have an outer segment filled with a dense stack of photosensitive membranes, an inner segment with high densities of mitochondria, a nucleus, and a synaptic region at the base (Fig. 3). Beyond this basic similarity, rods and cones differ structurally and functionally. Rods have long, slender outer segments whose photo-sensitive membranes are in disks separate from the plasma membrane. Cones typically have short, tapered outer segments formed by infolding of the plasma membrane. Cones mediate photopic vision, a term describing vision in bright light environments. Rods mediate scotopic vision, which describes vision in dim light environments. Additionally, several cone types of different spectral sensitivity underlie color vision (Marks 1965; Stell and Harosi 1976). Typically only one type of rod is present; thus rod-mediated color vision is not possible.
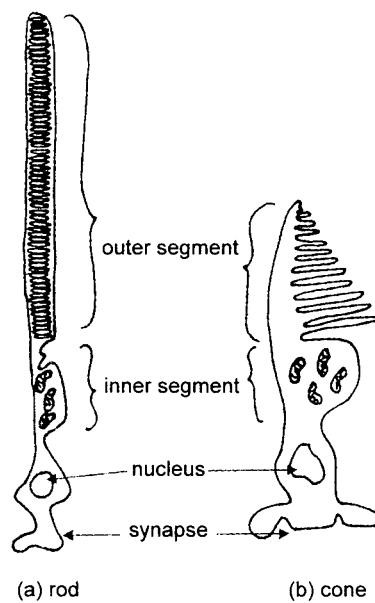
(a) rod   (b) cone

Fig. 3 Rod and cone photoreceptor neurons. The basic structure is illustrated for a) rod and b) cone cells. Outer segment membranes are the site of phototransduction. In rods the photosensitive membranes are in disks, separate from the external cell membrane; in cones the membranes are infoldings of the external membrane. The inner segments have many mitochondria.

Visual Pigments

Phototransduction is mediated by visual pigment embedded in the outer segment membranes of the photoreceptor cells (Applebury and Hargrave 1986). Visual pigments consist of opsin, an intrinsic membrane protein of approx. 350 amino acids (Nathans and Hogness 1984), plus a chromophore bound to the opsin (Kropf 1972). The chromophore absorbs light, whereas the opsin protein modifies the spectral absorbance of the chromophore. Rhodopsins have the chromophore 11-cis-retinal (vitamin A_1), while porphyropsins are based on 3,4 dehydroretinal (vitamin A_2). Rod and cone photoreceptors use vitamin A_1 and A_2 chromophores bound to the opsin, but both pigments function in a similar fashion (Stryer 1991). Using microspectrophotometry (MSP), fish visual pigments have been identified with spectral absorbance ranging from the far-red (Levine and MacNichol 1979) to the ultra-violet (Harosi and Hashimoto 1983). These differences in visual pigment spectral absorbance are attributed either to changes between vitamin A_1 and A_2 in the chromophore (Beatty 1975a), or to changes in the amino acid sequence of the opsin protein (Archer et al. 1995; Hope et al. 1997; Carleton and Kocher 2001).

Retinal Organization

The density and spatial arrangement of photoreceptors in the retina reflects the visual environment of a fish. A typical fish retina is made up of rods and cones distributed throughout the retina. Rods dominate the retinas of deep-sea and nocturnal fish (Wagner et al. 1998); cones dominate in larval stages and bright light fish (Collin and Collin 1988). Rods are often distributed unequally throughout the retina. In some fish, rods predominate in the dorsal retina (Kunz et al. 1985; Nag and Bhattacharjee 1989), in others they predominate in the ventral retina (Evans and Fernald 1993).

The density of photoreceptor cells determines the acuity and sensitivity of the retina. Acuity is the ability of the retina to resolve two objects; whereas sensitivity is the ability of the retina to capture photons at a given light intensity. Retinal acuity depends on the inter-photoreceptor spacing, the angle subtended on the retina by an image, and the convergence of cones onto ganglion cells (Fernald 1988). To increase visual acuity, cones are often closely spaced, with one cone for every ganglion cell. High densities of cones are typically observed in retinal areas involved with prey location (Browman et al. 1990; Shand et al. 2000). Even some deep-sea fish with an otherwise pure rod retina have an area of single cones in the region of binocular vision (Locket 1977; Munk 1989). To increase visual sensitivity rods are closely spaced, and many rod inputs converge on one ganglion cell. In deep-sea fish, these rods are often packed in bundles forming "macroreceptors" of varying cell numbers.

Cones also occur alone (as single cones), or in twos, threes and occasionally fours (as double, triple and quadruple cones). These multiple cones may function as macroreceptors, but the advantage they convey over single cones is unclear. Multiple cones are not found in the mammalian retina but are found in birds, amphibians, reptiles and fish (Cohen 1972). In the fish retina, cones are typically present in a repeating pattern, or "mosaic" of four double cones surrounding one central single cone (Fig. 4). In some species, an additional single cone type is
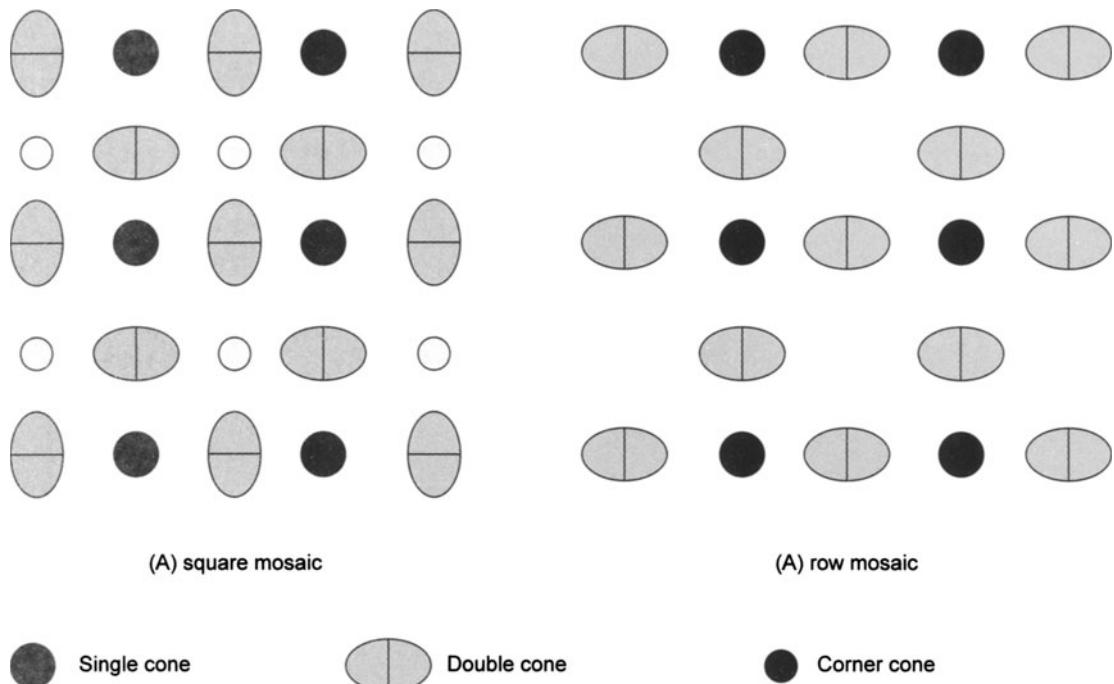
Fig. 4 Cone mosaic. The organization of typical cone mosaic is illustrated for a) square mosaic and b) row mosaic. (N.B. corner cones are not present in all species)

present at the corners of the mosaic (Engström 1963). Large rods have been observed in the cone mosaic but this is unusual (Collin and Collin 1998). The typical mosaic arrangement of single and paired cones is believed to be important for good visual perception, and fish relying on vision have well ordered mosaics. In contrast, nocturnal and deep-sea species do not have a cone mosaic (Engström 1963). For example, no cone mosaic is observed in the eel (*Anguilla anguilla*), or the stingray (*Dasyatis brevicaudata*) (Braekevelt 1984 1994). The sole (*Solea solea*), which relies on chemoreception, has double and triple cones, but no mosaic (Engström and Ahlbert 1963; Sandy and Blaxter 1980).

Regular cone mosaics are found in those animals feeding on fast moving prey, which suggests the mosaic is important for motion detection (Lyall 1957). A row mosaic is typical of fish from dim light environments (Engström 1963; Munk 1981 1990), and is thought to be sensitive to motion in two directions. A square mosaic is common in bright light species (e.g. Evans and Fernald 1993; Pankhurst et al. 1993), and may be important for tracking moving prey in all directions (Ahlbert 1973). Many fish possess regions of row and square mosaic in the same retina (Engström 1963; Ahlbert 1976; Beaudet et al. 1997). In some species, the mosaic first develops as a row mosaic and then transforms to the square mosaic (Ahlbert 1973). The cones can also alternate between row and square mosaic on a daily cycle (Kunz 1980; Wahl 1994).

Retinal Development

Although the adult retina varies dramatically between different species of fish, the embryonic development of the retina follows the typical vertebrate sequence. Ganglion, amacrine and horizontal cells form early in retinal development and photoreceptor cells form late (Sidman 1961; Blanks and Bok 1977; Carter-Dawson and LaVail 1976; Johns et al. 1979). In vertebrates, cone photoreceptors begin to differentiate well before rods (Mann 1964; Hollenberg and Spira 1973; Branchek and Bremiller 1984). Rods are added late in vertebrate neural development, and in fish, continue to be added throughout life (Johns and Fernald 1981; Johns 1982; Raymond 1985; Frölich et al. 1995).

As the fish eye continues its growth, the retina increases in size by addition of all cell types at the margin, a peripheral growth zone (Fernald 1989). As a result the fish retina displays a developmental gradient, with newly formed retina near the margin and the oldest retina at the center (Fig. 2). Rods are also continuously inserted into the central retina to maintain a constant rod density as the eye grows in size. These newly inserted rods arise from a population of rod progenitor cells in the outer nuclear layer (Johns and Fernald 1981). Another population of cells continues to proliferate in the inner nuclear layer (INL) (Julian et al. 1998). These INL cells appear to be multi-potent giving rise to the rod progenitors under normal growth conditions as well as cones during retinal regeneration (Wu et al. 2001).

The late appearance of rods correlates with the life history strategy of fish (Fig. 5; Evans and Fernald 1990). In species with *direct* development, rods appear shortly after cones during embryonic development (Hagedorn and Fernald 1992). Species with *indirect* development do not add rods until weeks or months after hatching (Sandy and Blaxter 1980). The one exception to the pattern may be the leptocephalus larvae of the Elopomorpha (Pfeiler 1986). The leptocephalus larvae of anguillid eels have a pure-rod retina (Braekevelt 1984; Pankhurst 1984); however, the retina of the earlier yolk-sac, larval stage of these species has yet to be examined.

In addition to the delayed appearance of rods, the expression of multiple cone pigments is delayed in fish retinal development. There also appears to be some variation in the sequence of visual pigment expression. In early developmental stages the retina is comprised entirely of single cones (Blaxter and Staines 1970; Evans and Fernald 1993; Shand et al. 1999). In species with a larval stage, such as the winter flounder, these larval single cones are green sensitive (Evans et al. 1993), but many marine fish larvae have green and ultraviolet sensitive single cones (Britt et al. 2001; Forsell et al. 2001). The adult single cones are typically blue-sensitive and the double cones are green and red sensitive (Levine and MacNichol 1979). In the goldfish and zebrafish, (species without a larval stage), the embryonic sequence of visual pigment expression is red, green, blue then ultraviolet (Raymond et al. 1995; Stenkamp et al. 1996). At varying developmental times, the larval or embryonic single cone lattice is transformed to a mosaic of single and double cones (Ahlbert 1973; Raymond et al. 1995; Shand et al. 1999). A current question is whether the expression of the adult cone pigments occurs before, during or after the formation of the cone mosaic. Although the transition from larval to adult visual pigments has yet to be demonstrated in metamorphic species; the evidence so far suggests that the pigments are expressed first and the mosaic is formed later (Stenkamp et al. 1996; Helvik
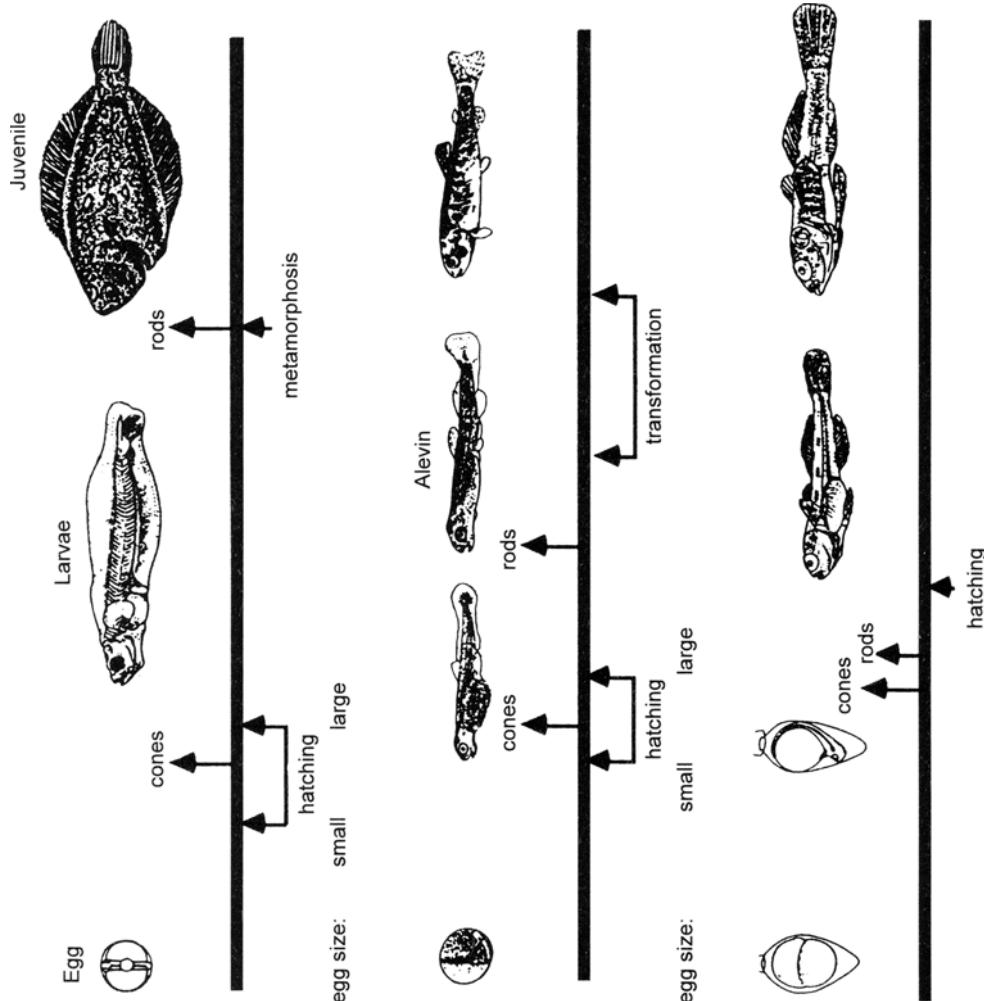
Fig. 5 Developmental strategies. The range of developmental timetables is illustrated for fish. Species with no larval stage are classified by direct development and the retina has all photoreceptor types near time of hatching. Protracted development with an extended larval stage is illustrated by the indirect strategy. In this case, cone photoreceptors arise close to time of hatching, but rods do not arise until metamorphosis. Variations between the two extremes are depicted by the intermediate strategy (after Evans, B.I. and Fernald, R.D. 1990).

et al. 2001). Thus, as the cone mosaic forms, the correct cone phenotypes must be arranged into their specific positions. What happens to the larval green and UV cones is not known, but further work is ongoing in this area.

The retinal cone mosaic of a typical adult teleost is a precisely oriented array of double cones and single cones. Mosaic formation requires some cells to form double cones while others remain single, a process which must involve significant regulation. Double cone formation in teleosts results from fusion of existing single cones (Ahlbert 1973; Sandy and Blaxter 1980), and the process has been documented using electron microscopy (Schmitt and Kunz 1988; Shand et al. 1999). During continued growth of the retina, new mosaic is formed at the germinal margins (Wagner 1975; Larison and Bremiller 1990), but in species with protracted (indirect) development, the cone mosaic initially arises from existing single cones (Fig. 6).
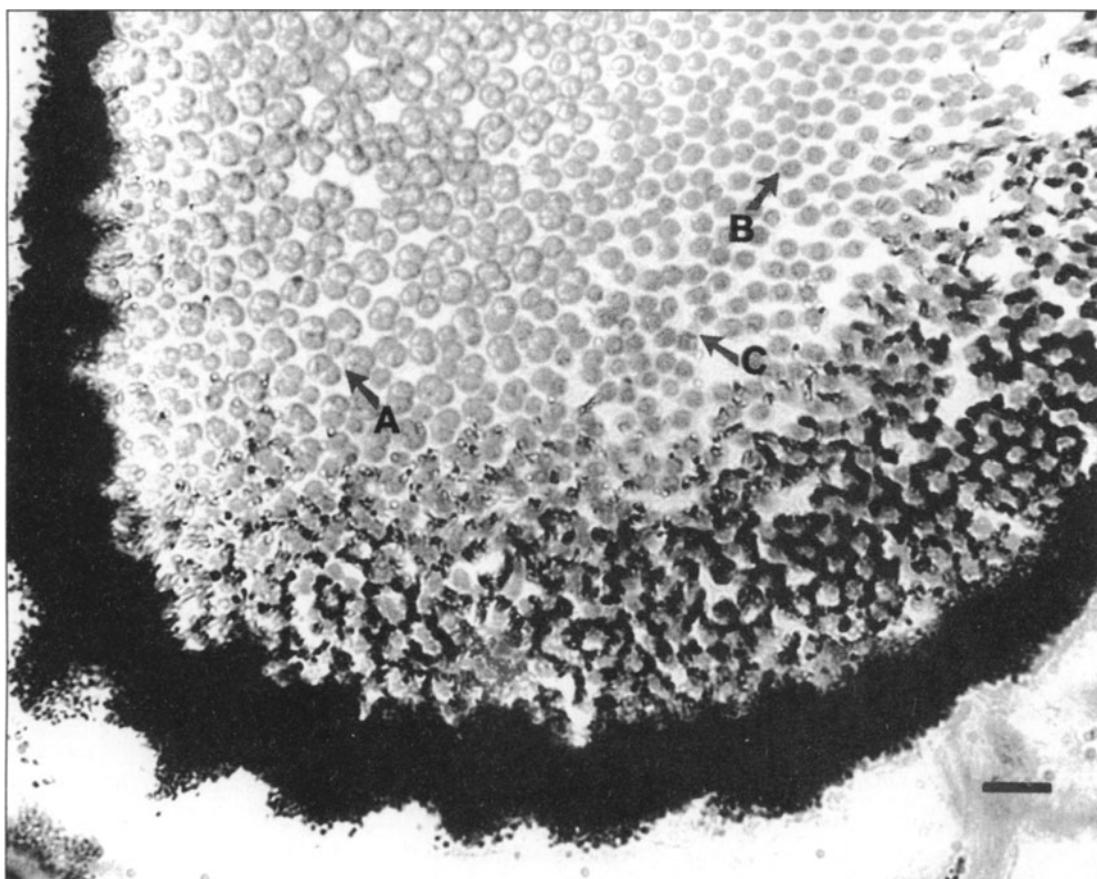
**Fig. 6** Transforming cone mosaic. Tangential section (3 microns) of metamorphosing winter flounder retina (65 dph). The cone mosaic has begun to form (A) but a region resembling the larval array is still present (B). Transitional region (C) shows aggregation of cells into the mosaic before fusion of double cones. The section shown here includes a region of the nasal retina central to the germinal margin. Scale bar 10 microns.

A gradient of developmental schedules for cone mosaic appearance is listed in Table 1. What determines when single cones transform to the mosaic of single and double cones? As outlined above, single cones typically arise first in the fish retina, and the timing of rod development can be predicted by the life history strategy (Evans and Fernald 1990); however, cone mosaic formation is independent of rod development (Wan and Stenkamp 2000). Rods are clearly important in scotopic (low light) vision and cone mosaics are implicated in motion sensitivity. Given the importance of the cone mosaic to visual performance (Ahlbert 1973), how does the timing of its formation impact the visual ecology of the fish?

Table 1

Species	Cone Mosaic Appearance
rainbow trout (*Oncorhyncus gairdneri*):	present at hatching (Schmidt and Kunz 1989)
zebrafish (*Brachydanio rerio*):	present at hatching (Raymond et al. 1995)
African cichlid (*Haplochromis burtoni*):	2 dph (Hagedorn et al. 1998)
goldfish (*Carassius auratus*):	2-3 dph (Johns 1982)
walleye (*Stizostedion vitreum*):	7 dph (Wahl 1994); 14 dph (Evans & Browman, unpubl.)
cichlid (*Tilapia leucosticta*):	7 dph (Grun 1975)
golden shiner (*Notemigonus crysoleucas*):	beyond 16-18 dph (Evans & Browman, unpubl.)
pike perch (*Lucioperca lucioperca*):	2-4 weeks post hatch (Ahlbert 1969)
perch (*Perca fluviatilis*):	22 dph (Ahlbert 1973)
sole (*Solea solea*):	5-6 weeks post hatch (Sandy & Blaxter 1980)
herring (*Clupea harengus*):	metamorphosis (Blaxter & Jones 1967)
black bream (*Acanthopagrus butcheri*):	40-55 dph (Shand et al. 1999)
winter flounder (*Pleuronectes americanus*):	55-60 dph (Evans and Fernald 1993)
Atlantic halibut (*Hippoglossus hippoglossus*):	80 dph (Kvenseth et al. 1996)

In the winter flounder, cone mosaic first appears at the end of the larval stage, coincident with "flexion" and the formation of caudal fin rays (Evans and Fernald 1993). Flexion is a developmental stage in many fish, where the notochord turns up in the apical lobe of the caudal fin. In the retinas of many larval Pacific Northwest fishes, double cones are rarely present prior to flexion (Britt et al. 2001). In rainbow trout, cone mosaic first appears at hatching (Schmitt and Kunz 1989), a time when caudal fin rays are forming in salmonids. The development of the fin rays in the zebra danio (*Danio rerio*; Fuiman and Webb 1988) and sea bream (*Sparus aurata*; Patruno et al. 1998) marks the beginning of a significant change in locomotor ability. At this time, the fish change from "eel-like" locomotion to caudal fin mediated locomotion. Development of the paired fins allows precise maneuvering of the head and body (Harris 1938). Controlling the position of the head is important to keeping images stable on the retina, and would complement the increased visual capabilities derived from the formation of cone mosaic. These events, flexion, fin ray development and cone mosaic formation are coincident in many species, and deserve further investigation.

ADAPTATION TO VISUAL HABITAT

In determining whether the fish eye is optimally adapted for the ambient light environment, a number of questions must be addressed. First, what intensities, wavelengths, and scattering of light are present in the environment? Second, do the ocular structures transmit this light to the retina? Finally, is the retina (photoreceptor pigment) sensitive to these intensities, wavelengths, and scattered qualities of light? Once these questions are answered, we can proceed to ask what advantages this sensitivity conveys to the fish.

Light Intensity

Adaptation to low light environment is reflected in mechanisms that increase sensitivity such as larger eyes and predominance of rods. In deep-sea fish, increased eye size is correlated with increased depth (Wagner et al. 1998), but there is a limit to this trend. Eventually increase in eye size is constrained by the body size of the fish and in many cases a tubular eye develops. Tubular eyes allow an increase in lens size without an increase in eye size. Such eyes have the optical advantages of a large eye, but are in proportion to the overall size of the fish (Marshall 1971). Below 1000 m in the ocean, there is insufficient downwelling light to form an image and eye size is again reduced. Adaptation to the extreme condition of total darkness is demonstrated by the blind cavefish (*Typhlogarra widdowsoni*), whose eyes are completely regressed (Marshall and Thines 1958). Degeneration of the retina is also observed in the parasitic fish, *Carapus mourlani*, which spends its adult stage inside a large species of starfish (Meyer-Rochow and Tiang, 1978).

Another response to light intensity is the relative proportion of rods to cones (Fig. 7). In high light environments, the retina has many cones (Collin 1988; Browman et al. 1990). As the light levels decrease, the retina becomes rod dominated (Meyer-Rochow and Klyne, 1982), and in most deep-sea fish, the retina is pure-rod (Wagner et al. 1998; Munk 1977). Many deep-sea fish have rod cells organized into bundles or banks which further increase light sensitivity (Locket 1977; Munk 1982; Kunz, et al. 1985; Es-Sounni et al. 1987; Frölich et al. 1995). Banks of rods are also observed in the torrentfish (*Cheimarrichthys fosteri*), a nocturnal, freshwater fish (Meyer-Rochow and Coddington, 2003).

Wavelength

Color of light and visual pigments vary dramatically among aquatic habitats (Fig. 7). An elegant correlation of visual pigments, environmental light and the behavioral ecology of fish is presented by Lythgoe (1984). Shallow, diurnal species have broad cone spectral sensitivity (400-600 nm); whereas the cone spectral sensitivity of deep-water or nocturnal fish is much narrower and shifted to longer wavelengths (530-620 nm). A similar trend occurs in the rod visual pigment sensitivity of freshwater fish. In shallow, diurnal species, rod sensitivity ranges from 505-520 nm as opposed to 525-545 nm in nocturnal or deepwater fish. In marine fish, visual pigments show adaptations to the spectra of downwelling sunlight and bioluminescence (Levine and MacNichol 1979; Crescitelli 1991; Douglas et al. 1998).
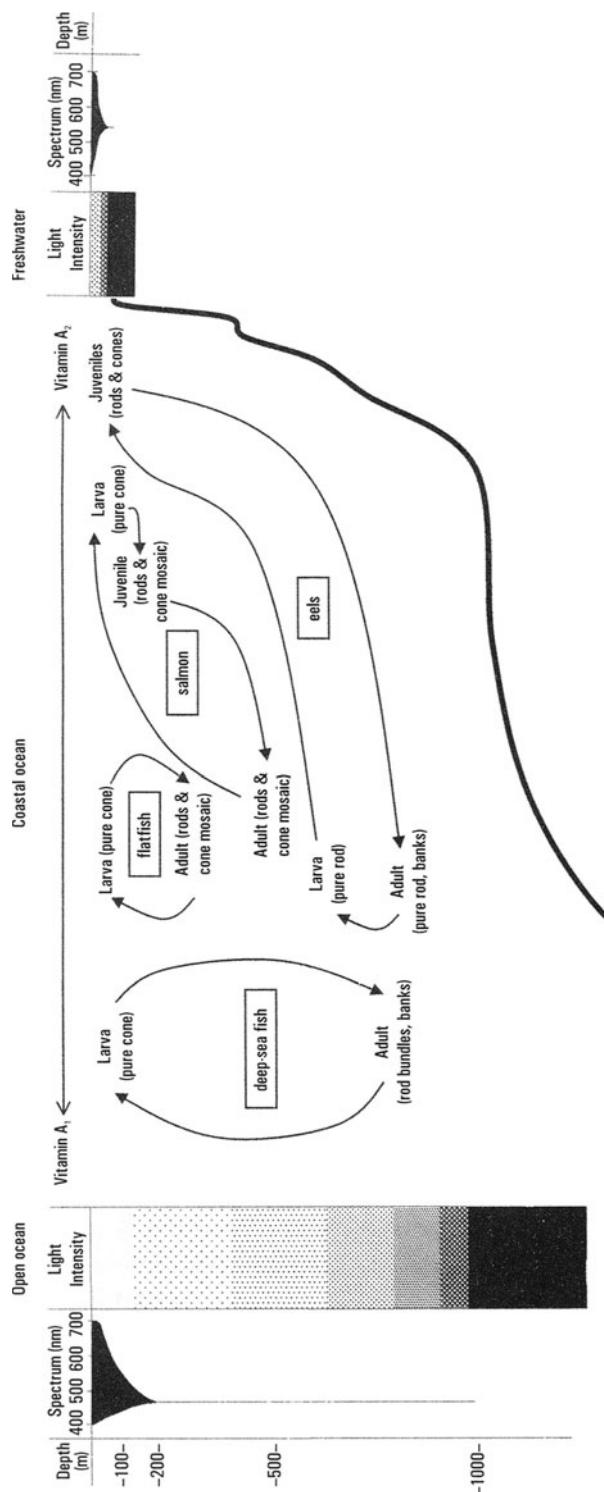
Fig. 7 Visual habitats and adaptations of the fish retina. The intensity and spectrum of available light are illustrated as a function of depth for the open ocean and freshwater habitats. Some corresponding adaptations of the fish retina to environmental light are depicted by species that migrate between these habitats during their life cycle. The chromophore of the visual pigment also changes between vitamin A_1 and A_2 as fish move between freshwater and marine environments.

In correlating visual pigment sensitivity and ambient light, two hypotheses are considered: (1) the "sensitivity" hypothesis and (2) the "offset" hypothesis (Lythgoe 1972). In the sensitivity hypothesis, visual pigment peak absorbance matches the predominant wavelength of ambient illumination. In the offset hypothesis, visual pigment absorbance does not match the background light, but is offset to increase visual contrast. In general, cones and deep-sea rod pigments match the ambient light conditions, whereas other rod pigments are offset (Loew and Lythgoe 1978; Lythgoe 1984).

An area of recent research activity involves the ultraviolet (UV) sensitive cones found in many species of fish. The first evidence of UV sensitive cones in fish was observed in the Japanese dace (Harosi and Hashimoto 1983). Since then UV cones have been found in many diverse species such as salmonids, halibut, goldfish and cichlids (Bowmaker and Kunz 1987; Browman and Hawryshyn 1994; Chen and Stark 1994; Carleton et al. 2000; Helvik et al. 2001; Forsell et al. 2001).

Ultraviolet light is transmitted by water and can penetrate to depths of 600m in clear water (Frank and Widder 1996). As a result, clear coral reef environments have relatively large amounts of UV light. Although UV light transmits readily through distilled water, it is quickly absorbed as dissolved organic compounds increase (James and Birge 1938 in Wetzel 1983). In coastal lakes, used by migrating salmon, UV cues are available only to a maximum depth of 18 m (Novales Flamarique et al. 1992). Although UV light is present in many fish habitats, the biological significance of UV sensitivity is under debate. The sensitivity hypothesis would predict that the presence of UV cones is a consequence of ultraviolet wavelengths in the environment. However, ultraviolet light does appear to have specific advantages, such as an increase in location distances to zooplankton prey by rainbow trout and pumpkinseed sunfish (Browman et al. 1994). UV cones also appear to have a separate neural pathway (Parkyn and Hawryshyn 2000), which may underlie a polarization sensitive visual system.

Polarized Light

Fish have demonstrated sensitivity to the e-vector of polarized light (Waterman and Forward 1972), and the response appears to be mediated by UV sensitive cones (Hawryshyn and McFarland 1987). A number of models for polarized light detection have been suggested. Double cone ellipsoids have been proposed to act as a light guide for polarized light (Cameron and Pugh 1991; Rowe et al. 1994), but these models do not account for the involvement of UV light. Additionally, no polarization sensitivity was observed in optic nerve recordings from post-larval sunfish with double cones (Novales Flamarique and Hawryshyn 1997). Another model suggests that the partition between the double cones acts to reflect polarized light to the adjacent UV-sensitive corner cones (Novales Flamarique at al. 1998). If true, a square cone mosaic, with UV sensitive corner cones, may act as an analyzer for the e-vector of polarized light. A variety of salmonids do exhibit different neural responses to horizontally versus vertically polarized light. Optic nerve recordings give maximal "off" responses only for horizontally polarized light; however, "on" responses are maximal for both vertically and horizontally polarized light (Parkyn and Hawryshyn 2000).

Given that fish may be capable of analyzing the e-vector of polarized light, how does this benefit the fish? What use does the animal make of polarized light information? There are a number of roles polarization cues could play for fish. Polarized light sensitivity may act to increase prey detection capability. Zooplankton are transparent but birefringent and become more visible when viewed by a polarization sensitive system. In fact, juvenile rainbow trout located zooplankton prey at greater distances under polarized light (Novales Flamarique and Browman 2001). Additionally, scattering within the water column produces horizontally polarized light, which degrades visual contrast. A vertical analyzer would reduce perceived scatter in the background and increase visual contrast (Wehner 2001). Detection of downwelling polarized light might also be used as a cue for migration (Kunz and Callaghan 1989). The degree of light polarization increases with distance from shore and could be used as an orientation cue (Schwind 1999). Although the final word on polarized light sensitivity in fish has not been established, there is compelling evidence that this is a valuable sensory modality.

Turbidity

Scattering of light by suspended solids creates turbid conditions in the water column. These suspended solids are usually silt and clay, but turbidity also results from high levels of phytoplankton or bacteria. Behavioral studies demonstrate that turbidity impacts vision in fish, but whether fish express any specific adaptations to turbid conditions is unclear. Given that turbidity limits visibility, one would predict that fish endemic to turbid habitats might show retinal adaptations. Such an example may be present in the goldeye, *Hiodon alosoides* which occurs in turbid rivers and has rod/cone bundles of 40-50 cells (Braekevelt 1982). In contrast to the pure-rod bundles of deep-sea fish, the photoreceptor bundles in the goldeye have equal numbers of rods and cones. The advantage of these rod/cone bundles for turbid water vision is not known. However, closely packed groups of photoreceptors (bundles) are found only in turbid water or deep-sea fish and may act as macroreceptors with a large area for photon capture (Locket 1977; Munk 1977).

Although turbidity decreases visibility, it may be beneficial to fish communities. Vinyard and O'Brien (1976) demonstrated that the reaction distance of fish to zooplankton prey is reduced in the presence of high turbidity, but this may actually help the fish. If encounter with predators is reduced, the result is beneficial to the prey and turbidity may protect the small fish from their predators. However, if encounters with prey or food supply are significantly reduced, the effect is detrimental to the forager. In the case of zooplankton, the reaction distances are short, such that turbidity may not completely eliminate their detection by fish.

To test the effects of turbidity on plankton feeding fish, community structure was monitored in two small experimental ponds (Evans and O'Brien unpubl). Both ponds were filled with identical zooplankton communities from a large reservoir pond. One pond was kept clear, whereas clay was added to the other pond to induce turbidity. Both ponds were stocked with an equal number of white crappie (*Pomoxis annularis*), a species that is planktivorous until a size of approx. 15 cm, when it becomes piscivorous. Over time, the condition (length × weight) of fish in the turbid pond increased while the condition of fish in the clear pond decreased. There were

incidences of spawning in both ponds; however, only in the turbid ponds did any young survive predation from larger conspecifics. Gut content analysis of the clear pond fish revealed few organisms, while guts from the turbid pond fish were packed with zooplankton. It would appear that by limiting predation, the turbid conditions prevented the predators from overexploiting the prey resources. Thus, for some species, turbidity appears to be a beneficial factor and may stabilize certain ecosystems.

Predator-Prey Adaptations

During their early life history, most fish feed on zooplankton and their success at foraging is key to their survival. However, before a prey item can be eaten, it must first be detected. The reaction volume and the amount of time spent searching determine the probability of prey detection by planktivorous fish (O'Brien et al. 1990). For a given prey type, the reaction volume (the visual field in which prey can be located), is determined by the location distance and the angle of the visual field. With large bodied prey, the location distance and visual angle are large, but with decreasing prey size the reaction volume decreases. Reaction volumes are even smaller under conditions of low light intensity or high turbidity (Vinyard and O'Brien 1976; Howick and O'Brien 1983).

Zooplankton take advantage of their reduced visibility in low light by undergoing vertical migration. At night, zooplankton are up near the water's surface feeding on phytoplankton. At dawn, as light intensity increases, zooplankton descend below the photic zone to reduce their risk of predation from fish (Blaxter 1976; O'Brien 1979, Lampert 1993). Larger bodied zooplankton descend deeper than small zooplankton. Thus, effective planktivores must be able to successfully forage on small-bodied zooplankton at low light intensity.

In order to increase search efficiency, planktivorous fish employ "saltatory search", a behavior which is intermediate between ambush and cruise search (Evans and O'Brien 1988; Anderson et al. 1997). Instead of continuously swimming while searching, planktivorous fish swim intermittently, making short repositioning runs separated by stationary search pauses (Fig. 1). Foraging can be divided into two categories: (1) prey detection and (2) unsuccessful search (when no prey are detected). The components of prey detection are: (a) the location distance to prey, (b) the angle of the visual field and (c) the time it takes the forager to detect prey in the visual field. What is most intriguing about saltatory search is that the components of unsuccessful search: (a) the repositioning distance, (b) the turn angles and (c) the duration of the search pause, match the values of prey detection. In other words, planktivorous fish vary their search behavior in accordance with the location parameters of previously encountered prey, matching their search behavior to their recent success in detecting prey.

Furthermore, when the size, species and densities of zooplankton change, plankton feeding fish make significant changes in their search behavior (Evans 1986). For a prey type of given detectability, the time devoted to an unsuccessful search is proportional to the time needed to detect these prey. Conspicuous prey, such as a 3-4 mm *Heterocope*, are easily detected by Arctic grayling (*Thymallus arcticus*). On average, if the grayling do not detect these prey items in 0.07

sec, they give up search and reposition. On the other hand, when searching for smaller zooplankton (0.5-1.5 mm), the fish wait an average of 0.3 sec before giving up and repositioning (Evans and O'Brien 1988). The same relationship is observed in sunfish, but the search pauses are of much longer duration (O'Brien et al. 1986). Many planktivorous fish require upwards of 1000 zooplankton prey in a day. Searches that are too long are a waste of time, yet searches that are too short might fail to detect available prey. By matching the duration of the search pause to an expected detection time, the fish use time very effectively.

In addition to changing search pause duration, planktivorous fish match the length of repositioning distance to location distance, and match turn angles during search to the visual angle in which prey were detected. As a result, a minimum amount of water is either searched twice or left unsearched. In the face of changing visual conditions, the ability to change search tactics allows fish to approach optimality in their foraging (O'Brien et al. 1989).

Few fish remain planktivores as they mature to adults, and most become piscivores. The most effective search strategy likely depends on the movement and abundance of prey. Many piscivores use ambush search whereas others may be closer to cruise search (O'Brien et al. 1990). Although saltatory search is well established for planktivores, it would be of interest to determine the degree to which piscivores incorporate the tactics of saltatory search into their foraging.

VISUAL SYSTEM RESPONSES TO ENVIRONMENTAL CHANGE

Changes in visually mediated behavior can increase the probability of detecting prey, but more extreme changes in the environment require structural changes in the visual system. In aquatic ecosystems, light conditions differ significantly between geographical locations, as well as on daily and seasonal cycles. In addition, during the course of their life history many fish migrate between habitats. Changes in retinal structure and function that occur in response to changes in the light regime provide strong evidence to support adaptation to habitat. The following discussion recognizes two categories of retinal change: (1) reversible change on daily or seasonal cycles; and (2) irreversible change such as the developmental transformations and metamorphic changes associated with movement from planktonic to benthic or deep sea habitats as individuals mature.

Reversible retinal change allows the fish to change retinal structure and function with changing conditions, but retains the flexibility to revert back to the initial state. These changes can be on daily, seasonal or migratory cycles such as those observed in anadromous and catadromous species.

Daily Change

Variations in light intensity cause retinomotor movements within the eyes of fish. Two types of retinomotor movements have been described: (1) the classic retinomotor movements of the pigmented epithelium and the photoreceptors towards and away from light (Blaxter and Staines 1970; Ali 1975); and (2) the more recently described twisting of the cone mosaic

between a row and a square mosaic (Kunz 1980; Wahl 1994). These movements allow the retina to be reconfigured to an optimal structure for each light condition.

The classical retinomotor movement of the pigmented epithelium and the rods and cones effectively provides fish with two functional retinas (Borwein 1981). During bright light, the retina is functionally all-cone for high acuity, whereas during dim light, both rods and cones are exposed for high sensitivity (Fernald 1989). At high light intensity, the cones contract away from the pigmented epithelium, and the rods are shielded from light by the pigmented epithelium. At low light levels, the opposite movements occur. Rods contract, the cones extend and the pigmented epithelium layer retracts, exposing both rods and cones to light. Such retinomotor movements do not occur in development until both rods and cones are present, and are not found in the pure-cone retinas of larval fish (Blaxter and Jones 1967; Blaxter and Staines 1970; Ali 1975). These movements occur on a daily basis in response to ambient light intensity and allow greater visual efficiency with changes in light intensity (Burnside et al. 1983).

The second type of retinomotor movement, the twisting movements of cone mosaic between row and square mosaic configurations, is also believed to increase visual capability under differing conditions (Wahl 1994). Row mosaics are more common in dim light and nocturnal species (Engström 1963), and the movement of the mosaic to a row configuration in dim light is compelling evidence for this function of the row mosaic (Kunz 1980). Reversion of the mosaic to a square configuration under high light intensity supports the idea that the pattern is beneficial under these brighter conditions. This intriguing phenomenon deserves more careful study.

Seasonal Change

Many environmental changes occur on a seasonal cycle. At higher latitudes, the winter sun is lower in the sky and spectrum of light is red-shifted. Many species of fish have combinations of vitamin A_1 and A_2 based visual pigments that change their relative proportions on a seasonal cycle (reviewed in Beatty 1975a). In the rudd (*Scardinius erythrophthalmus*), there is a change in the ratio of A_1/A_2 pigments in the photoreceptors, such that A_2 pigments predominate in winter, and A_1 pigments predominate in summer (Loew and Dartnall 1976). The use of vitamin A_2 pigments shifts the wavelength sensitivity of the photoreceptors to the longer wavelengths of ambient light.

Migratory Changes

Salmonids (Salmonidae) and eels (Anguilliformes) spend significant stages of their life history in both marine and freshwater environments. As they move between these environments, they encounter very different visual worlds (Fig. 7). Marine water has less dissolved organic matter such that ambient light is of shorter wavelength than in fresh water. Freshwater fish typically use vitamin A_2 based visual pigments for sensitivity to longer wavelengths of light, whereas marine fish use vitamin A_1 for shorter wavelength sensitivity. Both salmon and eels are

observed to switch the ratio of A_1 and A_2 pigments in their photoreceptors, with vitamin A_2 pigments predominating in freshwater and vitamin A_1 pigments predominating in marine habitats (Beatty 1975b).

Changes also occur in the UV sensitivity of salmonids as they migrate to the ocean (Bowmaker and Kunz 1987; Hawryshyn et al. 1989). The UV sensitive cones of salmonids are implicated in efficient foraging for zooplankton (Browman et al. 1994). However, as the fish approach smoltification and head for the ocean phase of their life cycle, these UV cones disappear (Novales Flamarique 2000; Deutschlander et al. 2001). Why these cones are lost is puzzling, especially because they seem to reappear when the fish return to freshwater at sexual maturity (Beaudet et al. 1997). It is unclear whether ecological or developmental factors prevail in this situation. Possibly the loss of the UV cones is a developmental by-product of hormonal changes (Novales Flamarique 2000). Loss of UV cells can be induced with thyroxine, a hormone that is also involved with smoltification (Browman and Hawryshyn 1994). There is also evidence that the UV cones are only lost in the ventral retina, with regions of the dorsal retina retaining UV sensitivity (Deutschlander et al. 2001).

In contrast to these reversible or cyclic changes in visual capabilities, many retinal changes are irreversible and are correlated with new stages in life history. Irreversible changes are associated with movement from planktonic to benthic or deep sea habitats, as individuals mature. Developmental changes in retinal structure and function can be termed transformations or metamorphoses depending on the rate of change. Transformation is a gradual change in structure and function; whereas metamorphosis is an abrupt change in features not related to reproduction. Metamorphosis occurs after an extended period of growth with little morphological change (Evans and Fernald 1990). Whether a developmental strategy is termed a metamorphosis or a transformation does not change the relevance of the morphological change. Instead, it indicates how abruptly an animal will change habitats. Those species that gradually move from one habitat to another can slowly change their retinal structure as they make the transition. In contrast, sharp divisions between habitats require animals to quickly change external morphology and visual capabilities to survive in the new environment.

Transformation

Virtually all fish are planktonic as larvae, switching to a new habitat as they become juveniles and adults (Fig. 7). The majority of these changes parallel a move from epipelagic to deeper water. A common transformation appears to be the loss of short wavelength sensitivity as the larvae move to deeper water (Shand et al. 1988; Chen and Stark 1994; Britt et al. 2001). UV sensitivity appears to be important to larval fish for detection of zooplankton prey and may be lost when fish leave the plankton and/or switch diets. It should be noted that not all these fish have corner cones in their retinal mosaic, so there may be two somewhat overlapping functions of UV sensitivity. All plankton feeders might benefit from UV sensitivity in their foraging, but only fish with UV sensitive corner cones in the square cone mosaic would have polarized light sensitivity (as per Novales Flamarique et al. 1998). In halibut larvae, the UV cones are only

found in the ventral retina (Helvik et al. 2001) which may be a region used for planktonic prey detection. As they mature, salmonids lose the ventral population of UV cones, which could be used for prey detection; retaining the dorsal UV-polarized light sensitive cones (Deutschlander et al. 2001).

Metamorphosis

More dramatic change is involved with fish that descend from surface waters to the deep sea. Many deep-sea fish have epipelagic larvae that metamorphose as they descend to the adult habitat. These larvae have pure-cone retinas that are later redesigned for low light conditions (Wagner et al. 1998). Eye shape is also redesigned with some fish developing tubular eyes at metamorphosis (Evans and Fernald 1990). Some deep-sea fish develop bioluminescent photophores at metamorphosis allowing them to be camouflaged against downwelling light (Anctil 1979).

Many fish undergo a true metamorphosis (reviewed in Evans and Fernald 1990), but perhaps the best known examples are the flatfish (Pleuronectiformes) and eels (Anguilliformes). Eels undergo two metamorphic events where both the body and the retina change dramatically. European eels (*Anguilla anguilla*) have an interesting life history (Barrington 1961). Eggs deposited in the Sargasso Sea hatch into yolk sac stage larvae (Pfeiler 1986). Given this remote location, not much is known about the visual system of the yolk-sac stage, but the later leptocephalus stage has a pure rod retina (Pankhurst 1984). Cones arise with metamorphosis to the glass eel stage (Braekevelt 1984), and appear to be important for the freshwater phase of the life cycle. With sexual maturity and return to the ocean, cones are again lost (Pankhurst 1982), and a pure rod retina with multiple banks of rods equips the adult eel for a dim light environment (Es-Souuni et al. 1987). The development of the pure rod retina of eels seems identical to that of other deep sea fish, whose larval stages have cones which are also lost during development of the pure rod retina (Wagner et al. 1998).

Metamorphosis in flatfish also involves an abrupt change in morphology. Larvae are transparent and planktonic, but after several months begin their metamorphosis to a benthic lifestyle. The body flattens and becomes pigmented on one side, and one eye migrates to join the other on the pigmented side of the head. Structural change parallels a change from an actively swimming larvae to a benthic adult that has cryptic coloration to avoid detection. Changes also occur in the flatfish retina at metamorphosis (Evans and Fernald 1993; Kvenseth et al. 1996; de Miguel Villegas et al. 1997). As in many marine teleosts, the larval retina is composed entirely of single cone photoreceptors with rod photoreceptors not appearing until metamorphosis (e.g. Sandy and Blaxter 1980; Evans and Fernald 1990). In addition, flatfish also delay the addition of specialized cone phenotypes and cone mosaic until metamorphosis (Evans and Fernald 1993, Kvenseth et al. 1996).

The formation of the cone mosaic is coincident with metamorphosis, but there may be some variation in the timing of expression of multiple cone pigments. Atlantic halibut larvae (*Hippoglossus hippoglossus*) possess mainly green sensitive single cones, but at the end of the yolk

sac stage (40 dph) they express mRNA for red and blue opsin throughout the single cone retina. UV opsin mRNA is also expressed, but only in the ventral retina (Helvik et al. 2001). In contrast, MSP measurements of winter flounder larvae, found only green absorbing cones throughout the larval period and no UV cones. After metamorphosis, winter flounder have blue sensitive pigment in single cones as well as red and green sensitivity in double cones (Evans et al. 1993). Although metamorphic species have been examined at various time points before and after metamorphosis, there has still not been demonstration of how the change in the structure of the retina and the expression of various opsins are co-ordinated. With the advances of *in situ* hybridization for retinal opsin mRNA, the answer should arrive soon. Given that UV opsins have been found in turbot and halibut larvae (Forsell et al. 2001) it is curious that none were detected in the winter flounder. Possibly the coastal habitat of winter flounder has limited UV light such that these larvae would not benefit from UV sensitive cones.

Metamorphic changes in the visual system are abrupt changes in structure and function. Prior to metamorphosis, the eye and retina are of a constant morphology and grow in this state until a critical period when they change to a new morphology. Many transformations occur in normal development, but metamorphic change coincident with moving to a new habitat is particularly intriguing. The fish eye has many similarities to the human eye and is a useful model for understanding vertebrate retinal development. The variety of fish retinas adapted to specific habitats allows pure cone or pure rod retinas to be investigated in natural systems. In contrast to higher vertebrates, the fish eye continues to grow throughout the life of the animal. Presumably this allows fish the developmental flexibility to modify their sensory structures as they move to different visual environments. The transformations that occur within the visual system of fish offer a unique chance to observe developmental change in the context of environmental change.

Despite the extensive literature available on vision in fish, there are many areas that still need further investigation. The mechanism underlying polarized light detection and the significance of UV and polarization sensitivity in fish deserves further consideration. Another intriguing feature of the fish retina is the cone mosaic. It would be of interest to establish the importance of this configuration to foraging behavior, and to investigate the correlation between fin ray and cone mosaic formation. Along these lines, investigating the pattern of onset of adult cone visual pigments in concert with cone mosaic formation is another valuable area of work. Further work should also be encouraged to understand the function of row versus square cone mosaic, and the movement between these configurations as a function of ambient light intensity. Given the ultimate need for vision as a tool within the life of a fish, there is a need for continued investigation of retinal structure and function in the context of prdator-prey interactions and search behavior.

Acknowledgements

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The Photoreceptors and Visual Pigments of Sharks and Sturgeons

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ABSTRACT

With the exception of the stellate sturgeon, which has only cones, all acipenseriform species studied have a duplex retina. The retina is dominated by rods, but about 20% of the photoreceptors are cones. The rods are structurally similar to those of vertebrates in general. Most rods have outer segments that are broad and long, but at least one species has a very rare, very slender rod as well. Cones are also typical in structure, and they too are rather robust. With one exception, the inner segment of each cone houses a colorless oil droplet, the function of which is unknown. Packing density of both rods and cones is relatively low, indicating that both scotopic and photopic acuity is less than that of other nocturnal creatures. The visual pigment in the rods of all species studied has a peak absorbance near 540 nm. All species studied have multiple cone pigments and, therefore, the photoreceptor basis for wavelength discrimination. However, one cannot yet say whether or not any sturgeon or paddlefish has color vision. All visual pigments are based on the vitamin A_2 chromophore, and there is no evidence that there is a shift to the vitamin A_1 chromophore with change of habitat or with age. At least one species does change its cone pigment complement with age. Larval white sturgeon up to 10 weeks of age have only green-sensitive cones; after 10 weeks, blue-sensitive and red-sensitive cones are also present.

Sharks also have a duplex retina with both rods and cones. The cone population varies from *ca.* 1% in bottom dwelling species to *ca.* 20% in diurnal predators that feed near the surface. The rods are typical but much thinner than those of the acipenseriforms. Cones are also typical and are also relatively small. Peak absorbance of shark rod pigments varies from 472 nm to 502 nm and appears to be correlated with depth of habitat. No absorbance measurements have been made from shark cones, so the nature of the cone pigment or pigments is unknown. It is also not known if any species of shark has color vision. One species, the lemon shark, shifts its rod visual pigment from a vitamin A_2 form in the juvenile to a vitamin A_1 form in the adult, a shift correlated with a change in the spectral environment of the habitat. Otherwise, all shark visual pigments thus far studied are based on vitamin A_1.

Key words: Paddlefish, Photoreceptors, Shark, Sturgeon, Vision, Visual Pigments

INTRODUCTION

Although there are certain species of fishes whose behavior is not highly dependent on the visual sense (the blind cave fish serves as an extreme example), fishes as a group find the ability to analyze their photic environment extremely valuable. That this is so is made obvious by several simple observations of morphology and behavior. For example, many if not most fishes have relatively large and well developed eyes. Many species are highly colored and many of these are sexually dimorphic, a definite indication that color plays an important role in mating display and mate choice. Many species are patterned in such a way as to confuse the vision of other, predatory, fishes. Some species, such as the archer fish or trout, which strike at insects above the water surface, obviously employ vision in their feeding behavior.

This obvious dependence on the visual sense, coupled with the tremendous variety in the photic environments inhabited by different species, have made the fishes a favorite group for study by investigators interested in relating an animal's visual capability to its behavior and the ecological niche that it occupies (e.g., Meyer-Rochow and Coddington 2003). In fact, the fishes, more than any other group, have contributed to our understanding of the relationship between the light-absorbing characteristics of the photosensitive visual pigments housed within the rod and cone photoreceptors and an animal's habitat or life-style. Certainly, the fishes have been, by far, the greatest source of evidence in support of the 'sensitivity hypothesis', which holds that the visual pigments within the photoreceptors evolved so as to match their absorbance to the spectral character of the light in the environment (e.g., Crescitelli 1991). This relationship between visual pigment absorbance and the spectral environment is particularly important because the simple absorbance of a photon is, of course, the very first event that then sets into motion all of the extremely complex biochemical and physiological mechanisms that ultimately lead to visual perception.

Almost all of our knowledge of the photoreceptors and visual pigments of fishes has come from the study of modern teleost species. However, in recent years a fair amount of information has been garnered from other, non-teleost fishes, notable among which are the sturgeons and the sharks. These fishes have been studied, in part, because they are 'primitive', in the sense that they have a very long phylogenetic history. They have been studied also because of their generally interesting behaviors and, particularly in the case of certain species of sharks, their interactions with man and other mammals. It is, therefore, to the photoreceptors and visual pigments of the sturgeons and the sharks that this chapter is devoted.

STURGEONS AND PADDLEFISH

A Duplex Retina: Rods and Cones

The order Acipenseriformes includes 25 living species in two families, the Acipenseridae or sturgeons and the Polyodontidae or paddlefishes. Every species studied thus far, save one, has been shown to have a duplex retina. That is, the retina contains both rods, the highly sensitive photoreceptors responsible for vision under dim-light or scotopic conditions, and cones, the

less sensitive photoreceptors responsible for more acute vision under bright-light or photopic conditions, as well as for wavelength discrimination or color vision. The photoreceptors in the retina of the white sturgeon, *Acipenser transmontanus*, are shown in Fig. 1A; those in the retina of the shovelnose sturgeon, *Scaphirhynchus platorynchus*, and the paddlefish, *Polyodon spathula*, are shown in Figs. 1B and 1C, respectively (Sillman et al, 1990, 1999).
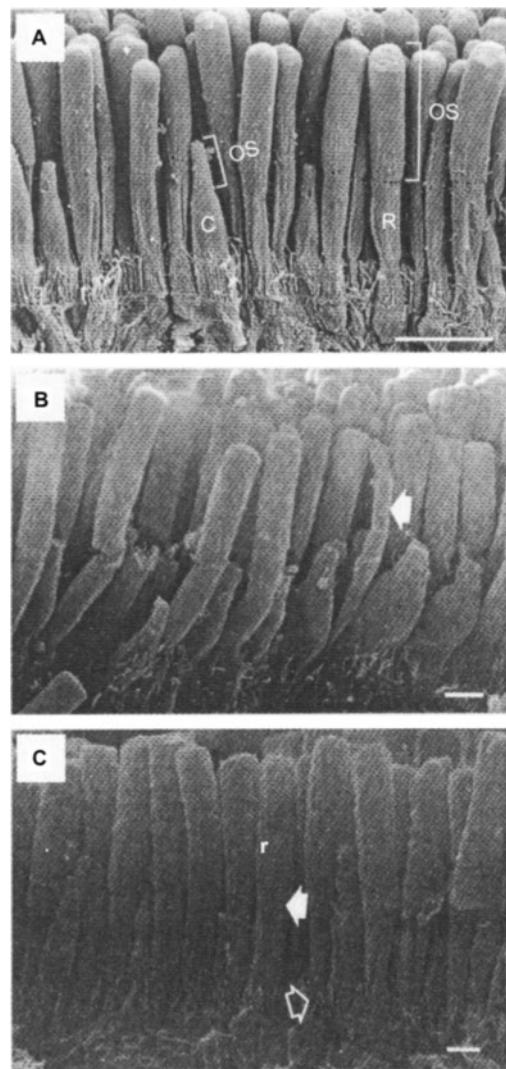

Fig. 1 Scanning electron micrographs of photoreceptors of white sturgeon (**A**), shovelnose sturgeon (**B**) and paddlefish (**C**). In **A**, OS denotes the outer segment of a cone (C) and a rod (R). The filled arrow in **B** points to the rare, narrow rod. In **C**, the filled arrow points to a calyx; the open arrow to a Müller cell process; r denotes a rod. Scale bars are 15.0 μm in **A**, 5.0 μm in **B** and **C**. **A** is from Sillman et al. (1990); **B** and **C** from Sillman et al. (1999).

For the most part, sturgeon and paddlefish rods are typical vertebrate rods. That is, the outer segment of the cell, that region of the cell that contains the photosensitive visual pigment, is a relatively long, cylindrical structure that extends from an inner segment of similar diameter. Numerous calycal processes or calyces extend from the distal portion of the inner segment onto the outer segment. The function of these calyces is not known, although they must, at the very least, serve to support the outer segment whose connection to the inner segment is quite delicate. The rods in all three species shown are large and their dimensions are really quite similar, although the outer segments of the paddlefish rods are a bit longer (ca. 23.7 μm) and a bit broader (ca. 5.6 μm) than are the outer segments of either the white sturgeon (ca. 19.5 × 4.7 μm) or the shovelnose sturgeon (ca. 18.5 × 4.2 μm).

The rods alluded to above are the only type of rod found in either the white sturgeon or the paddlefish retina. In the shovelnose sturgeon retina, however, a second, very rare type of rod is present (white arrow in Fig. 1B). This rod is typical in its general conformation but, with a diameter of about 2.3 μm, it is much narrower than the common rod. Only two such rods were observed, and it is likely that they comprise less than 1% of the total rod population in the shovelnose sturgeon. This narrow rod is so rare that one cannot say with certainty that they are not also present in other acipenseriform retinas and were simply missed with the scanning electron microscope. As applied to the retina, scanning electron microscopy is, after all, essentially a sampling technique. Multiple rod morphotypes have not been reported before in any species of fish, but they have been described in other animals. For example, some nocturnal snakes and *Sphenodon* are known to have more than one type of rod in their retinas (Walls 1942). Some amphibians also have two types of rods in their retinas, and they are known to be both morphologically and functionally distinct (Walls 1942; Denton and Wyllie 1955; Sillman 1987). The less common amphibian rod, which comprises about 10% of the population, contains a visual pigment with peak absorbance near 430 nm whereas the more common rod is most sensitive near 500nm. Thus, the 'secondary' rod of the amphibian retina provides the animal with greater sensitivity to blue light under light conditions where cones are not operating. Unfortunately, we were never able to record from, or even identify, the narrow type sturgeon rod with the microspectrophotometer and, therefore, its function remains a mystery.

Structurally, all sturgeon (and paddlefish) cones appear to be similar, and only single cones are present. Fish retinas often contain cones that are joined tightly together or have multiple outer segments, so called twin, double or triple cones (e.g., Miyazaki et al. 2002; Meyer-Rochow and Coddington 2003), but such a cone has never been reported in a sturgeon retina. Most sturgeon cones are rather robust as cones go, with a rather large, globose inner segment from which extends a tapering outer segment. As with the rods, calyces extend from inner to outer segment in the cones.

Cone Oil Globules

With one exception, all sturgeon cones contain an oil globule in the inner segment. Because of the way that tissue is treated in preparation for examination with the SEM, this globule cannot be seen in scanning electron micrographs. However, they are quite apparent in the light

micrograph in Fig. 2. Govardovskii et al. (1991) did report the presence of an unusually small, single cone lacking an oil droplet in the retina of the Siberian sturgeon, A. *baeri*, but such a cone was not seen in the shovelnose sturgeon, white sturgeon or paddlefish retina. The oil globules differ in size from cone to cone, but we have not yet been able to make a correlation between the size of a cone's oil globule and its light sensitivity. The sturgeon retina is not unique in having cones that contain oil globules. Birds and turtles, for example, also have cones with oil globules in their inner segments. However, whereas the oil globules of diurnal birds and turtles are highly colored, absorbing strongly in the shorter wavelengths, the oil globules in sturgeon cones are colorless. That is, sturgeon oil globules exhibit no wavelength-specific absorbance between 350 and 750 nm. Since the cones, as is true of all the photoreceptors, are oriented distally in the retina such that their photosensitive outer segments are 'pointing' towards the sclera, the oil globule sits in the light path. It is a certainty, therefore, that the colored oil globules of diurnal birds and turtles, which absorb visible light, have an important influence on the light sensitivity of the cones and, therefore, on wavelength discrimination or color vision. Since such colored oil globules preferentially absorb the shorter wavelengths, they would also serve to increase acuity by reducing chromatic aberration and glare. Since the oil globules in sturgeon cones do not absorb light across the visible spectrum, it is not clear what their function is. It is interesting that animals with colorless oil globules tend to be nocturnal or live in environments devoid of bright light. For example, although all diurnal birds have vividly colored oil globules in their cones, the oil globules in owl cones are colorless (Walls 1942). It would seem, then, that the adoption of life in a dark or dim light environment where sensitivity is most important and high acuity is secondary is consistent with the presence of colorless oil globules. Sturgeons and paddlefishes are, for the most part, bottom feeders who spend most of their time in a dim light environment. Perhaps diurnal ancestors of the extant acipenseriforms had colored oil globules in their cones, and the colorless oil globules are merely an evolutionary remnant, however, it is impossible to say.
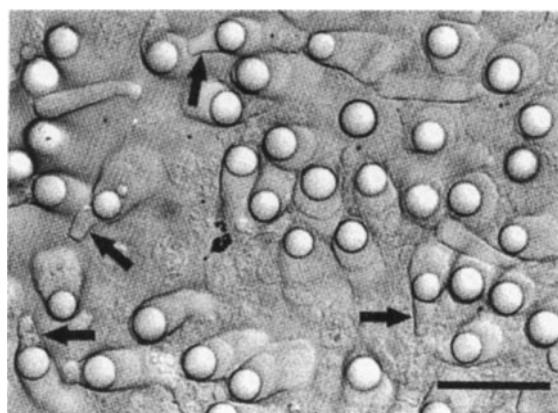

Fig. 2   Light micrograph of white sturgeon cones showing the oil globules housed in the inner segments. Arrows point to cones with relatively intact outer segments. Scale bar is 25.0 μm.

One must be careful not to carry the association between colorless oil globules and nocturnality too far. After all, the sturgeons do have a significant percentage of cones in their retinas and, as we shall see below, the cone population is characterized by multiple cone pigments. It is reasonable to assume that photopic vision is at least of some importance. In fact, one sturgeon species, the stellate sturgeon (*A. stellatus*), is reputed to have a retina that contains only cones (Govardovskii and Zueva 1987). Such a retina is certainly associated with vision under bright light conditions and is characteristic of animals with a diurnal habit and behavior that depends heavily on the visual sense. It is notable in this respect that whereas other sturgeon species appear to be consistently benthic in habit, the stellate sturgeon often swims in the middle and upper water layers where the lighting would be more intense and, therefore, cones would be more useful. Although stellate sturgeons are generally at the water's bottom at night, they are frequently found in the upper water layers during daylight hours (Shubina et al. 1989).

Photoreceptor Distribution and Packing Density

To obtain accurate values for rod and cone packing densities, and to determine whether or not the acipenseriform retina might have areas of specialization where either the rods or the cones are present in unusually high concentration, whole mounts were prepared from the retinas of the white sturgeon. Thus, the retina was removed from the eye, isolated from its pigmented epithelium, and placed on a glass slide, photoreceptor side up. Several radial cuts were made to flatten the otherwise concave tissue and a cover slip, supported in such a way as to prevent crushing of the tissue, was placed over the retina. The retina was mapped using a Nikon Eclipse Model E600 microscope and Scion Image video-enhancement software. At a magnification of 200X, images 0.69 × 0.53 mm were captured and archived so that the entire retina could be reconstructed after the cell counts were made. Such an image, taken from a cone preparation, is shown in Fig. 3A; one from a rod preparation is shown in Fig. 3B. Within each image,
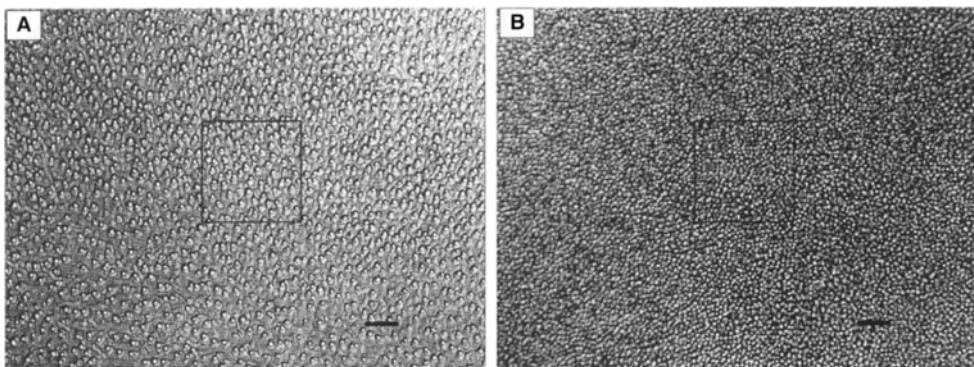

Fig. 3   Light micrographs showing a cone preparation (**A**) and a rod preparation (**B**) used for mapping the retina of the white sturgeon. The boxed area in each frame is the area in which cells were actually counted. Scale bars are 50.0 μm.

photoreceptor densities were determined by outlining a 0.15×0.15 mm square (e.g., boxed area in Fig. 7A and 7B) and counting the rod outer segments or cone oil globules within that 0.0225 mm^2 area. Each 0.0225 mm^2 area sampled was marked to enable location of its exact position after the retina was reconstructed by reducing each 0.69×0.53 mm image and 'pasting' all the resized images together. An example of a completely reconstructed retina is shown in Fig. 4. The data from two retinas were then superimposed and, using the optic disk as the central point,
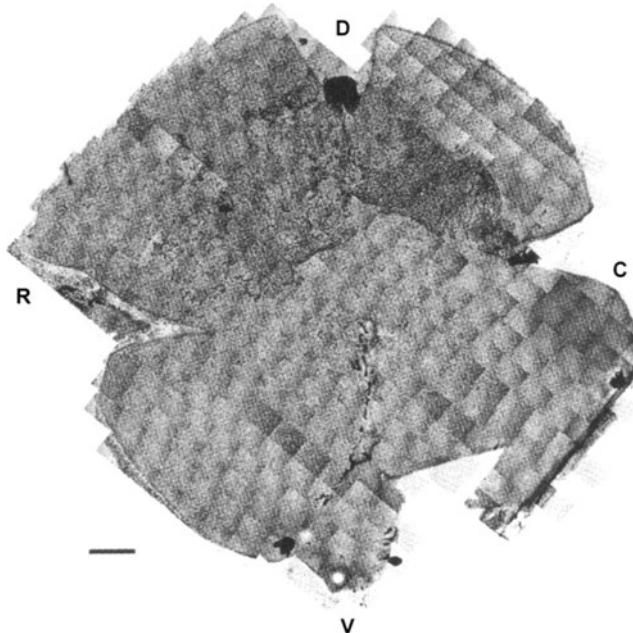
Fig. 4   Light micrograph showing a white sturgeon retina completely 'reconstructed' from frames such as those shown in Fig. 3. D denotes dorsal; V ventral; R rostral; C caudal. Scale bar is 1.0 mm.

quadrants and radii (at 1.0 mm intervals) were delineated, as shown in Fig. 5. The numbers scattered throughout Fig. 5 represent photoreceptors per mm^2 rounded off to the nearest thousand. Each is placed at the exact spot on the retina where that count was made. Composite data were compared first by analysis of variance (ANOVA), after which T-tests were used to determine the significance of differences between specific regions. Other than a tendency for the photoreceptors to be less densely packed at the far periphery, no region of the retina appeared to be significantly different from any other region. Some idea of the data can be gained from Table 1, where cone and rod densities are shown for each quadrant, for the dorsal and ventral halves, and for the entire retina. The absence of a specialized region of photoreceptors is consistent with the early work of Walls (1942) who described the sturgeon retina as lacking such a region. However, it is somewhat at odds with the study of Ito et al. (1999) who found a relatively high concentration of ganglion cells along the horizontal plane in the retina of the white sturgeon.
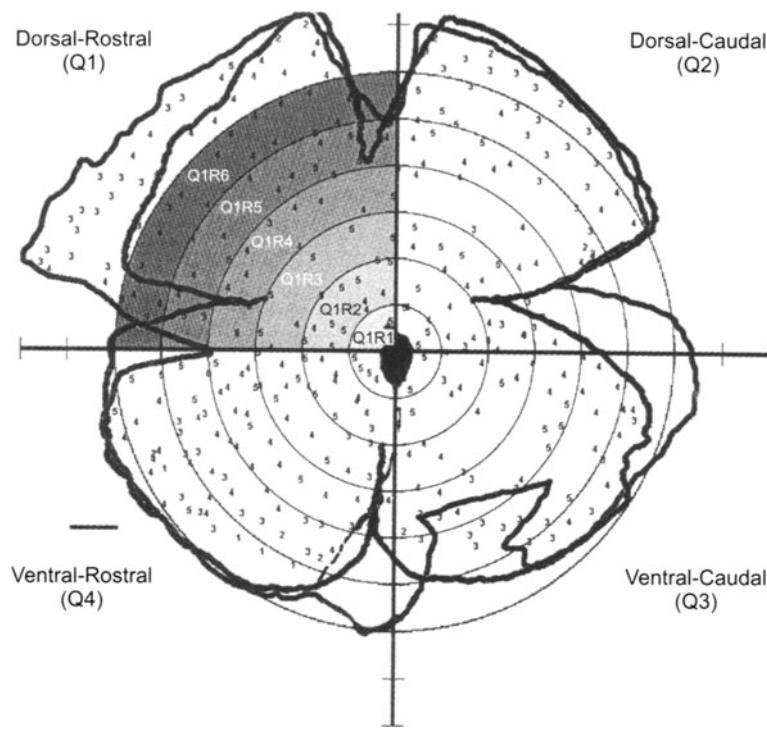
Fig. 5   Two white sturgeon retinas superimposed and divided into quadrants (Q) and radii (R). The numbers represent cone densities rounded off to the nearest 1000, and are placed at the exact spot where the counts were taken (e.g., boxed area in Fig. 3). Scale bar is 1.0 mm.

Mean rod density for the entire retina is 15,329 +/- 2,679 rods/mm^2, whereas mean cone density for the entire retina is 3,855 +/- 832 cones/mm^2. Thus, cones comprise 20% of the total photoreceptor population in the retina of the white sturgeon. These values are substantially lower than previous estimates (Sillman et al. 1990; 1999) made from studies with the scanning electron microscope (SEM). However, there is little doubt that the values obtained with the

Table 1   Photoreceptor densities in white sturgeon retina

Retinal region	Rods/mm^2 +/- s.d. (n)		Cones/mm^2 +/- s.d. (n)	
Dorsal-rostral quadrant	14,518 +/- 2,693	(58)	4,054 +/- 750	(108)
Dorsal-caudal quadrant	13,444 +/- 2,979	(38)	3,746 +/- 868	(77)
Ventral-rostral quadrant	16,387 +/- 1,788	(45)	3,822 +/- 913	(93)
Ventral-caudal quadrant	17,016 +/- 1,496	(43)	3,683 +/- 733	(58)
Dorsal retina	14,093 +/- 2,843	(96)	3,926 +/- 814	(185)
Ventral retina	16,694 +/- 1,673	(88)	3,769 +/- 848	(151)
Entire retina	15,329 +/- 2,679	(184)	3,855 +/- 832	(336)

retinal whole mount preparation described here are the more accurate. For one thing, substantial tissue shrinkage results from the dehydration procedure during preparation of the retina for analysis with the SEM, and previous estimates did not take that into account. For another, one can sample only relatively few regions of the retina with the SEM, whereas the whole mount preparation allows one to count all the photoreceptors throughout the retina. Moreover, there is a definite tendency when producing SEM micrographs of the retina to choose regions that are rich in cones, since those cells are of particular interest. Nevertheless, the previous conclusion that the acipenseriform retina is dominated by rods but contains a substantial complement of cones still holds.

To put things into perspective, it is interesting to compare rod and cone packing density with other animals that are active in a relatively dim light environment, especially animals for which we might have a 'feel' with respect to how they use their eyes. For example, the rods of the cat are much more densely packed at 310,000 - 485,000 rods/mm^2 (Steinberg et al. 1973). So too are the rods of the North American opossum at 216,000 - 478,000 rods/mm^2 (Kolb and Wang 1985), the owl monkey at 216,000 - 478,000 rods/mm^2 (Ogden 1975) and the rat at 374,000 - 400,000 rod/mm^2 (Cone 1963; Mayhew and Astle 1997). The conclusion that sturgeons have comparatively poor visual acuity and poor visual sensitivity under scotopic conditions would seem inescapable. Similarly, the opossum, with 8,000 cones/mm^2 in its specialized area centralis, and the cat, with 27,000 cones/mm^2 in its area centralis, would certainly have better visual acuity than the white sturgeon under photopic conditions as well.

The Visual Pigments

Virtually all of our knowledge about the light absorbing characteristics of acipenseriform visual pigments has been gained with the microspectrophotometer (MSP) which enables us to measure absorbance in single photoreceptors. Typical MSP absorbance curves are exemplified by the white sturgeon data shown in Fig. 6. The data for all species examined are summarized in Table 2, where the wavelength of maximum absorbance or λ_{max} is noted. With the exception of *A. stellatus*, which has no rods, all species studied thus far have rods which contain a visual pigment absorbing maximally in the green region of the spectrum. All species, without exception, have a cone (LWC) that is maximally sensitive to relatively long wavelengths. Three species also have middle- wavelength-sensitive cones (MWC) and short-wavelength-sensitive cones (SWC); two have only the MWC in addition to the LWC, and one has only the SWC in addition to the LWC.

The interesting thing here is that each of these fishes has a multiple cone pigment system and a retina with substantial numbers of cone photoreceptors. This type of retina is ideal for wavelength discrimination or color vision and, therefore, the question immediately arises as to whether or not the sturgeons and paddlefishes have that capability. To date, there is no published report that specifically addresses this question, however, there are some data that do touch on the issue. For example, there is a remark in a paper by Burkhardt et al. (1983) to the effect that they were able to identify luminosity-coded horizontal cells (integrative retinal neurons which receive input from photoreceptors) in the retina of the shovelnose sturgeon, but
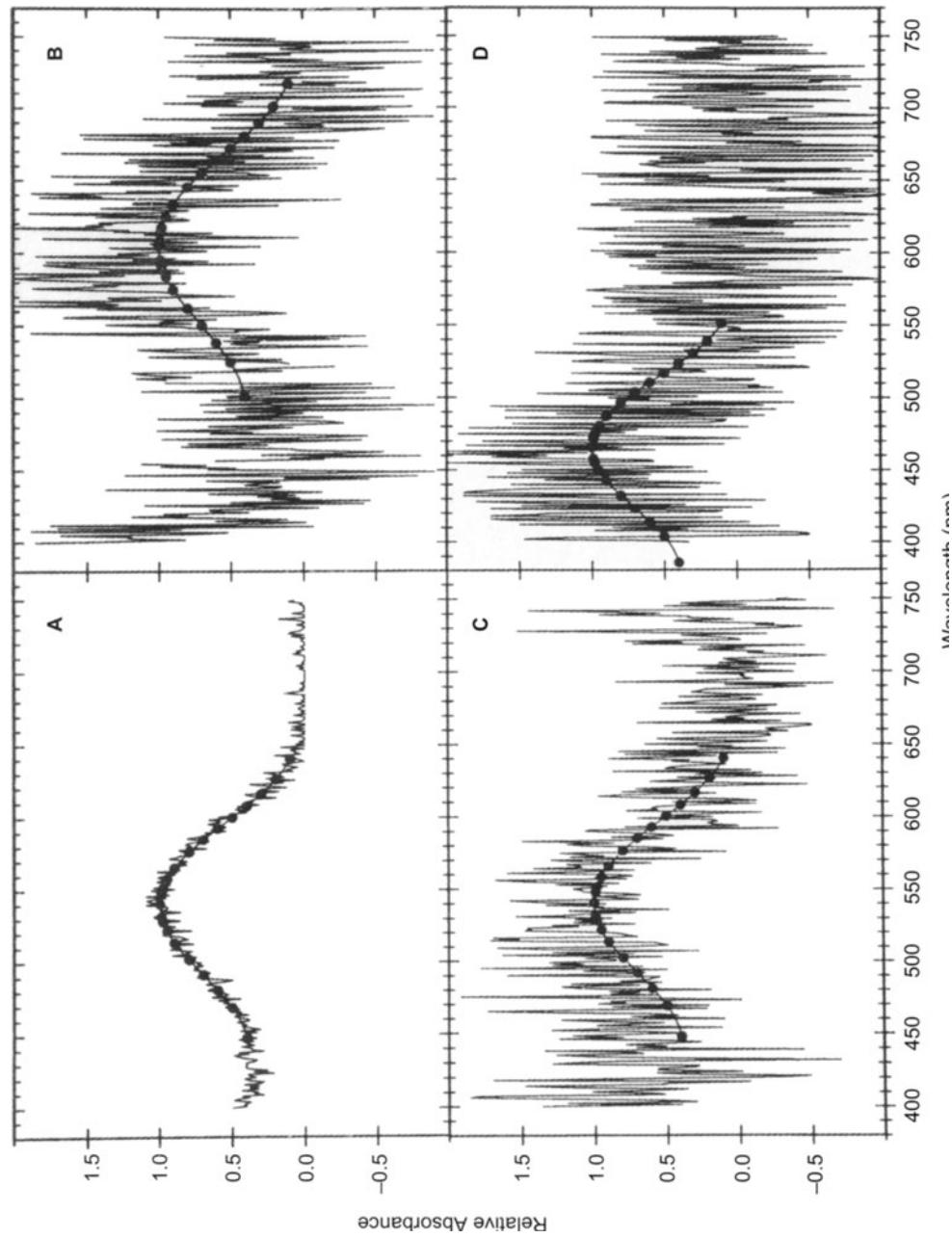
Fig. 6 MSP absorbance curves recorded from photoreceptors of the white sturgeon. The filled circles and broken line overlaid on each record is the best fit nomogram curve for a vitamin A_2-based visual pigment. (**A**) Rod. (**B**) Red-sensitive cone. (**C**) Green-sensitive cone. (**D**) Blue-sensitive cone. From Sillman et al. (1995).

Table 2 Sturgeon and paddlefish visual pigments and the photic environment

Species	Peak Absorbance in nm				Highly Frequented Depth in m (oceanic)	Dominant Wavelength in nm	Habit
	Rod	LWC	MWC	SWC			
Polyodon spathula Paddlefish	540[1]	607	535		n.a.	Varies; generally reddish[8]	Rivers; river lakes; feed on zooplankton.[10]
Scaphirhynchus platory-nchus Shovelnose sturgeon	534[1]	610	521	470	n.a.	Varies; generally reddish[8]	Rivers; river channels; feed on aquatic insect larvae.[11]
Acipenser transmontanus White sturgeon	540[2]	605	531	464	to 100[5]	580 to 590 in ocean[9]; varies, but generally reddish in rivers[8]	Semi-anadromous; rivers to coastal ocean; bottom feeder.[11]
Acipenser baeri Siberian sturgeon	549[3]	613	549	465	<50[6]	Varies; generally reddish[8]	Some semi-anadromous; rivers to estuaries and deltas. Some fresh water. Bottom feeder.[6]
Acipenser stellatus Stellate sturgeon		573[4]		455	3 to 300[7]	580 to 590 in ocean[9]; varies, but generally reddish in rivers[8]	Anadromous; rivers to coastal ocean; bottom at night; upper layers during day.[7]
Acipenser ruthenus Sterlet	545[4]	617	542		n.a.	Varies; generally reddish[8]	Rivers; sometimes brackish water; bottom feeder.[12]

[1] Sillman et al (1999)
[2] Loew and Sillman (1993)
[3] Govardovskii et al (1991)
[4] Govardovskii and Zueva (1987)
[5] Doroshov (2002). Personal communication.
[6] Sokolov and Vasil'ev (1989a)
[7] Shubina et al (1989)
[8] Lythgoe (1979)
[9] Dartnall (1975)
[10] Russell (1986)
[11] Conte et el (1988)
[12] Sokolov and Vasil'ev (1989b)

they were unable to find horizontal cells that were coded for chromaticity. Although this remark was merely made in passing and, in any case, negative data are always somewhat unconvincing, these data would indicate that the shovelnose sturgeon does not have the capacity to discriminate wavelengths. Similarly, and consistent with the results of Burkhardt et al. (1983), Govardovskii et al. (1991) cited Orlov (1966) as examining A. *stellatus* with a color substitution technique and finding no evidence for color vision. In contrast, however, Govardovskii et al. (1991) did find chromaticity coded horizontal cells present in the retina of the Siberian sturgeon, A. *baeri*, making the conclusion that this species has color vision a reasonable one. Nevertheless, the question of whether or not acipenseriforms in general have the ability to see color must await a more concerted effort to examine these animals both behaviorally and with electrophysiological techniques.

It is important to recognize that the development of a multiple cone pigment system would be advantageous to an animal even if not associated with the ability to discriminate wavelengths. There is good evidence from molecular biology that multiple cone pigment systems appeared very early in evolution (Nathans et al. 1986; Okano et al. 1992; Johnson et al. 1993; Hisatomi et al. 1994) and, therefore, it is certainly not surprising to find such a system in the retinas of 'ancient' fishes such as the sturgeons and paddlefishes. As opined by Sillman et al. (1999), the early adaptive value of the multiple pigment system probably was not color vision, but rather was the consequent increase in sensitivity across a spectral range much broader than is possible with a single pigment, as well as the enhanced ability to detect contrast (Lythgoe 1979). Both a broader spectral sensitivity and enhanced contrast detection would be of great importance to the sturgeons and paddlefishes, which spend much of their time in a spectral environment where all wavelengths of light are quite limited. It seems likely that the relatively complex proximal mechanisms that are associated with wavelength discrimination would have evolved only after the multiple cone pigment system was in place.

The Role of Vision in Acipenseriforms

This brings up the question of just what is the importance of the visual system to sturgeons and paddlefishes. It is certainly not likely that these fishes would have evolved and retained their relatively well developed eyes with their well developed duplex retinas if vision were not somehow important to their behavior. But exactly what that importance is remains unclear. Several studies indicate that the visual sense is not the dominant sensory modality employed during feeding behavior in either paddlefishes (Wilkens et al. 1997; Wilkens this volume) or sturgeons (Sbikin 1974; Lindberg 1988). This is not surprising since these fishes are primarily bottom feeders operating in an environment where you might well expect vision to be de-emphasized. Nevertheless, this is not to say that vision plays no role in feeding; it might very well play a supplementary rather than a dominant role. Certainly the visual sensitivity implied by the presence of many relatively tightly packed, large rod photoreceptors would be of value in behaviors other than feeding. Even in a dim light environment, the retina of the sturgeons and paddlefishes would prove useful in detecting differences in light intensity, and that ability would be important for orientation and for the detection of potential predators. We know, for example,

that the larvae of A. *transmontanus* are quite responsive to changes in light intensity (Loew and Sillman 1998). That is, under conditions of darkness the larvae move up into the water column where they swim almost continuously. Under very dim light, swimming is interrupted by periods of drifting, while exposure to bright light inhibits swimming completely and the larvae drop back down to the substrate where they take cover. This light mediated behavior pattern would be of great value for dispersal of the larvae while decreasing the risk from predation, since a motionless object will be of less interest to a predator than a moving object. Interestingly, the action spectrum for this negative photokinesis matches quite closely the absorption spectrum of both the green-sensitive rods and the middle-wavelength-sensitive cones and, therefore, either of those photoreceptor types could be involved in the behavior.

Age-related Changes in the Visual Pigments

The spectral absorbance curve is simply a graphic representation of the relative efficiency with which a visual pigment captures photons of a given wavelength. The position of the curve on the wavelength axis is a function of the interaction between the protein, or opsin, portion of the visual pigment molecule and the prosthetic group or chromophore to which the opsin is bound. Even a single change in the amino acid sequence of the opsin can have a major effect on the position of the absorbance curve. In fact, for the most part, the absorbance characteristics of a visual pigment are determined by the opsin's amino acid sequence. However, the nature of the chromophore is also very important in determining the λ_{max}. Although most visual pigments incorporate a chromophore based on vitamin A_1, many incorporate instead a chromophore based on vitamin A_2, where there is an additional double bond in the ring structure. The importance here is that a visual pigment with the vitamin A_2-based chromophore will have an absorbance curve shifted toward longer wavelengths, when compared to a visual pigment having the identical opsin but incorporating the vitamin A_1-based chromophore. For example, a vitamin A_1-based pigment with λ_{max} at 508 nm would have a vitamin A_2-based counterpart with λ_{max} at 541 nm (Whitmore and Bowmaker 1989).

Although certainly not a universal truth, in general the photoreceptors of freshwater fishes contain visual pigments based on the vitamin A_2 chromophore whereas the visual pigments of fishes that inhabit a marine environment incorporate the vitamin A_1 chromophore. Moreover, the photoreceptors of fishes that migrate between fresh and salt water often contain both vitamin A_1- and vitamin A_2-based visual pigments, with the dominant type determined by the aquatic environment, that is, A_2-based pigments dominating when in fresh water and A_1-based pigments in greater concentration when in salt water. Presumably, this state of affairs is a reflection of the fact that fresh water is generally redder than ocean water (Lythgoe 1979; Bowmaker 1990). This is of interest here because, in the broadest sense, all sturgeon species are migratory, although between species there is a great deal of variability in the pattern of migration (Bemis and Kynard 1997). All species spawn in fresh water. Some, such as the green sturgeon (A. *medirostris*), are fully anadromous. That is, after attaining a certain size, juvenile green sturgeons migrate from the river to the sea, only returning to fresh water years later as sexually mature adults intent on spawning. Potamodromous species, such as the shovelnose sturgeon and the

paddlefish, migrate not between the river and the sea but between different regions of the freshwater environment in which they spend their entire lives. Seemingly intermediate between these two types are species, such as the white sturgeon, that can best be described as semi-anadromous. That is, although juvenile white sturgeons do migrate to the sea, adult white sturgeons can be found in the river at any time of the year and, therefore, do not limit their travel up the river solely to the purpose of spawning.

Somewhat surprisingly, no species of sturgeon or paddlefish studied thus far, regardless of migratory pattern, has been found to have visual pigments that incorporate the vitamin A_1 chromophore. Of course, almost without exception, the fishes studied have been cultured and maintained in freshwater where, in the native habitat, the vitamin A_1 chromophore would be fostered. To see if life in saline water would make a difference, Sillman et al. (1995) examined the retinas of several white sturgeons caught in an estuary near San Francisco Bay. The estuarine water was indeed quite saline, ca. 673 mosmol. compared to *ca.* 909 mosmol. in nearby coastal ocean water and no measurable osmolarity in samples taken from the Sacramento River. The retinas of these wild sturgeons yielded no trace of a visual pigment based on vitamin A_1; their visual pigments were virtually identical to those of cultured white sturgeons bred and reared in freshwater tanks. Although provocative, these results are, unfortunately, still not conclusive in establishing the vitamin A_2 chromophore as the sole type in the photoreceptors of Acipenseriformes, since white sturgeon are not *fully* anadromous and, as noted above, adults can be found in the river at any time of the year. Several green sturgeons are currently being maintained in the sea water tanks at the UC Davis Bodega Marine Laboratory located north of San Francisco, and we hope examination of those fishes will produce a definitive answer.

Although there is, as yet, no evidence that the chromophore of acipenseriform visual pigments changes with age, there is evidence that the visual pigment complement of at least one sturgeon species does change with age. That is, Loew and Sillman (1993) found that white sturgeon larvae up to 10 weeks of age did not have the short- or long-wavelength-sensitive cones that are present in older individuals. They had only the rod pigment and the middle-wavelength-sensitive cone pigment, both of which absorb maximally between 530 nm and 540 nm. Thus, either could be responsible for the phototactic behavior mentioned above. Although it is an obvious, and reasonable, assumption that there is important adaptive value in adding visual sensitivity in the blue and red regions of the spectrum as the white sturgeon ages, what that adaptive value might be is not clear. Certainly, the feeding habits of white sturgeon larvae are much different from those of older individuals but, as noted above, acute vision does not appear to play a vital part in feeding. Both the larvae, which feed on zooplankton at night, and the adults, which feed on the muddy bottom, feed under conditions of very low illumination where the importance of vision is not apparent. Nevertheless, it is likely that there is some important, visually dependent behavior in the adults that is related to the spectral environment. It is interesting that there is no similar age-related change in visual pigment complement in either the shovelnose sturgeon or the paddlefish (Sillman et al. 1999). This might very well reflect the fact that these two species are strictly freshwater and the habitat of the adults is not very different from that of the larvae.

SHARKS

As a group, the sharks are much more successful than the sturgeons, with 368 species of living sharks having been described (McFarland 1991). The ecological niches that they occupy vary tremendously. Some inhabit the great depths of the oceans, while others live in shallow coastal waters, and still others frequent estuaries and even rivers. Some species are migratory and travel vast distances, possibly for reproductive reasons, whereas others reside in relatively restricted waters year round. Some species are plankton eating filter feeders, while others are bottom feeders preying primarily on invertebrates, and a few are at the very top of the food chain, preying on large mammals such as elephant seals, sea lions and the occasional human. Some species appear to be active only under conditions of very dim illumination, whereas others are active during the light of day when they seek prey at the surface, and still others are active both diurnally and nocturnally. Such great variation in behavior and habitat implies an equally great variation in visual capability, thus making the photoreceptors of the shark an interesting and important object of study.

The Photoreceptor Population: Cones as well as Rods

Despite early reports of the presence of both cones and rods in the retinas of certain species of shark (e.g., Walls 1942), a view of sharks as visually deficient, all-rod animals prevailed until the work of Gruber et al. (1963), who firmly established the presence of a substantial population of cones in the retina of the lemon shark, *Negaprion brevirostrus*. Since then, numerous studies have confirmed that at least 17 species of shark, from various photic environments, have duplex retinas containing both rod and cone photoreceptors (Gruber and Cohen 1985), and so it seems likely that most, if not all, sharks have duplex retinas. Both the rods and cones of the shark are structurally similar to vertebrate photoreceptors in general, as described above, although compared to the acipenseriforms both cell types are substantially more narrow (Figs. 7 and 8). For example, rod outer segment diameter in both the brown smoothhound, *Mustelus henlei*, and the leopard shark, *Triakis semifasciata*, is about 2.8 μm, and cone outer segment diameter at the base is between 1.0 and 1.4 μm. These dimensions are consistent with those reported by Gruber et al. (1975) for seven other species of shark. If we correct for shrinkage the values obtained by Sillman et al. (1996) with the SEM, we can approximate packing densities of 66,000 rods/mm^2 in both the brown smoothhound and the leopard shark.

The fact that the retinas of the sharks studied thus far are all duplex in nature does not mean that their photoreceptors are identical in either structure or distribution. There is certainly a great range with respect to the ratio of rods to cones. Cones are very rare in some species, such as the brown smoothhound shark and the leopard shark, where they comprise, at best, 1% of the photoreceptor population (Sillman et al. 1996). In contrast, the central retina of the white shark, *Carcharodon carcharias*, contains 20% cones (Gruber et al. 1975; Gruber and Cohen 1985). This difference undoubtedly reflects the lifestyles of these sharks. Whereas the brown smoothhound and leopard sharks are primarily bottom feeders foraging along the muddy and murky substrate, the white shark is an active, diurnal predator whose hunting strategy is

highly dependent on the visual sense (McCosker 1985). The lemon shark, an animal known to be active during both day and night but considered to be more nocturnal than the white shark, has a retina with about 8% cones (Gruber and Cohen 1985). Moreover, both the white shark and the lemon shark, as well as several other species, appear to have specialized retinal regions that are particularly rich in cones, emphasizing the importance of acute vision to these predators (Gruber and Cohen 1985; Hueter 1991). Structural adaptations may also reflect differences in behavior. For example, Gruber and Cohen (1985) suggested that the longer length of lemon shark rod outer segments, as compared to white shark rod outer segments, is a reflection of the greater degree of nocturnality of the lemon shark. Certainly a longer outer segment, where the photosensitive visual pigment resides, would be a more efficient light trap and better suited for a nocturnal habit. Similarly, Sillman et al. (1996) suggested that the brown smoothhound shark either might be more nocturnal or might frequent greater depths than the leopard shark, since the rod outer segments of the former are substantially longer than those of the latter (30.6μm vs.
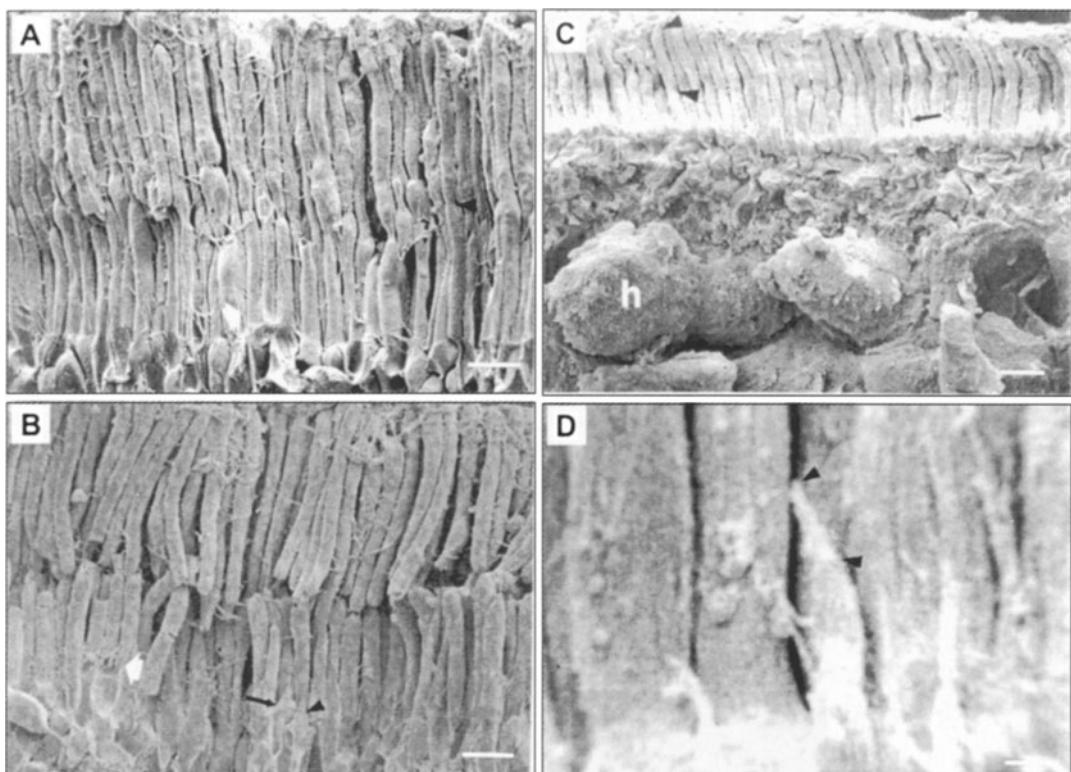
Fig. 7   Scanning electron micrographs of the photoreceptors of the brown smoothhound shark (**A** and **B**) and the leopard shark (**C** and **D**). White arrows in **A** and **B** point to Müller cell processes. Black arrow in **B** points to a cone; black arrowhead to the outer segment of another cone. Black arrowheads in **C** delineate a rod outer segment; black arrow points to a cone; h denotes a horizontal cell. **D** is a photographic enlargement of the cone in **C**; black arrowheads delineate the outer segment. Scale bars are 10.0 μm in **A**, **B** and **C**; 1.0 μm in **D**. From Sillman et al. (1996).
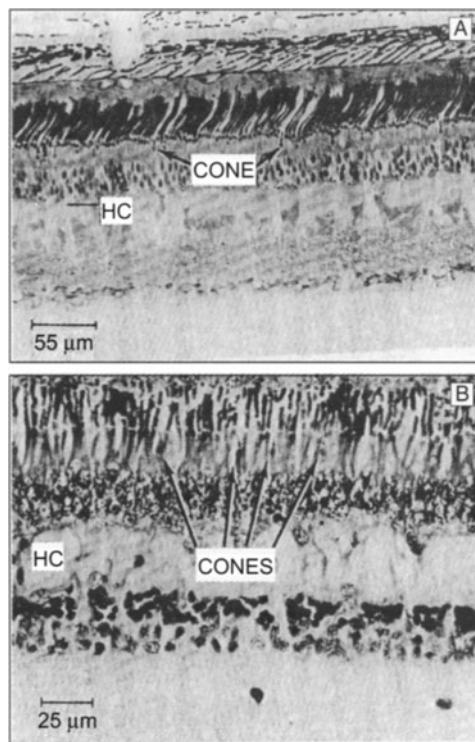
Fig. 8 Light micrograph showing the photoreceptors of the lemon shark (**A**) and the white shark (**B**). HC denotes horizontal cells. From Gruber and Cohen (1985).

19.3 μm). These suggestions make sense for two reasons. First, there is evidence that animals can adapt to a dim light environment by, at least in part, developing longer rod outer segments (Schremser and Williams 1995). Second, there appears to be a correlation between the depth at which a shark is active and the length of its rod outer segments (Kohbara et al. 1987). Although, unfortunately, we do not know much about the behavior of either the brown smoothhound or the leopard shark, the possibility that the brown smoothhound frequents greater depths is consistent with the fact that the peak absorbance of its rod pigment is at a shorter wavelength than that of the leopard shark, as we shall see below.

The Visual Pigments

To our knowledge, there has been no published report of a successful MSP recording of absorbance from a cone photoreceptor of any shark species and, therefore, the visual pigment complement of shark cones is unknown. McFarland (1991) suggested that the white shark, which generally strikes from below at prey on the surface, would have a cone containing a visual pigment with peak absorbance near 500 nm, since such a pigment would "provide a broad match to down-welling light near the surface of both oceanic and coastal seas and, thereby,

enhance the contrast of silhouetted prey." This suggestion, however, obviously must remain a speculation until such time as hard data become available. Unfortunately, such data are extremely elusive. Sillman et al. (1996) was unable to find leopard shark or brown smoothhound shark cones with the MSP. This is not surprising since the cones of these species are very few in number and quite small as well. Greater success would undoubtedly be had with sharks, such as the white shark, whose retinas contain substantial numbers of cones. However, for obvious reasons, it would be extremely difficult to obtain fresh, dark adapted tissue for laboratory examination. It is likely that knowledge of the absorbance characteristics of shark cone pigments will come eventually from studies using immunohistochemistry or DNA/protein expression analysis.

Since we do not know if multiple cone pigments are present in any species of shark, we cannot say whether or not some sharks have the basic retinal mechanism necessary for wavelength discrimination. Ultimately, of course, color vision can be proven only by a well controlled behavioral study, and no such study on sharks has been published. Electrophysiological studies on the lemon shark showed that selective chromatic adaptation did not result in a change in the photopic spectral sensitivity curve . This implies that the lemon shark retina contains only a single type of cone and, therefore, forces the conclusion that the lemon shark does not have color vision (Cohen 1990). It would be rather risky, however, to extend this conclusion to sharks in general.

In contrast to the complete lack of data regarding absorbance in shark cones, a fair amount of information is available regarding the visual pigments of shark rods. This is so primarily because rod pigments are easily extracted for analysis by traditional spectrometry, and also because the relatively large size and high numbers of rods make them suitable for either *in situ* or MSP analysis. With one exception, discussed below, all shark rod pigments examined thus far are similar in that they are all based on vitamin A_1 (Crescitelli 1991). However, Table 3 shows that there is a good deal of diversity in the absorbance characteristics of shark rod pigments. Peak absorbance of these vitamin A_1-based pigments ranges from 472 nm to 502 nm (Bayliss et al. 1936 reported a λ_{max} of 505 nm for *Scyliorhinus canicula*, but photoproduct interference makes that value unreliable). The blue shifted pigments are characteristic of those species that spend all or much of their time at great depths, whereas the pigments with peak absorbance closer to 500 nm are found in those species that inhabit shallower waters. For example, the specimens of *Centroscymnis coeliolepsis*, with a 472 nm pigment, were brought up from a depth of 1150 meters (Denton and Shaw 1963). In contrast, the leopard sharks that yielded a rod pigment with peak absorbance at 502 nm were taken from the shallow coastal water of Tomales Bay in northern California. It is most interesting that the swell shark, *Cephaloscyllium ventriosum*, has two rod pigments, one with peak absorbance at 478 nm and the other at 498 nm. During the summer, this species migrates to the surface from its usual habitat depth of about 450 meters Crescitelli (1991). Although the issue of the spectral quality of the light that actually reaches a shark's eye is a truly complex one, involving such factors as line of sight, time of day, particulate matter, etc., there is no doubt that ocean waters become bluer with depth (McFarland 1991). It seems highly likely, therefore, that the blue shifted rod pigments represent an adaptation to life in deeper

Table 3 Shark visual pigments and the photic environment.

Species	Peak Absorbance (λ_{max}) in nm	Highly Frequented Depth in m	Dominant Wavelength in nm[11]	Habit
Centroscymnus coeliolepis Portuguese dogfish	472[1]	668 to 3675[7]	465 to 470	Bottom dwelling.[12]
Cephaloscyllium ventriosum Swell shark	478 498[2]	to 450[2] <40 (summer)	465 to 470 580 to 590	Bottom dwelling; mostly nocturnal.[8]
Centrophorus squamosus Leafscale gulper shark	482[1]	400 to 750[7]	465 to 470	Bottom dwelling.[12]
Apristurus brunneus Brown catshark	483[2]	174 to 952[8]	465 to 470	Bottom oriented.[8]
Deania calcea Birdbeak dogfish	484[1]	475 to 894[7]	465 to 470	Usually on or near the bottom.[7]
Squatina californica Pacific angel shark	488[2]	<15 - 183[8]	580 to 590	Nocturnal bottom dweller.[8]
Mustelus henlei Brown smoothhound	496[3]	Shallows[8]	580 to 590	Inshore waters bottom dweller.[8]
Squalus suckleyi [acanthias] Piked dogfish	498[4]	Shallows to 183[9]	580 to 590	Highly migratory; usually coastal but sometimes oceanic.[9]
Mustelus californicus Gray smoothhound	499[2]	2 to 46[8]	580 to 590	Inshore waters bottom dweller.[8]
Scyliorhinus canicula Smallspotted catshark	500[5]	Shallows to 100[10]	580 to 590	Usually muddy or sandy bottom of inshore waters.[10]
Negaprion brevirostris Lemon shark (adult)	501[6]	<40[8,13]	465 to 470	Very clear near shore waters.[13]
Triakis semifasciata Leopard shark	502[3]	4 to 7[8]	580 to 590	Inshore waters bottom dweller.[8]

[1]Denton and Shaw (1963), [2]Crescitelli (1991), [3]Sillman et al (1996), [4]Beatty (1969), [5]Denton and Nicol (1964), [6]Bridges (1965), [7]Compagno et al (1989), [8]Castro (1983), [9]Lineweaver and Backus (1970), [10]Pizzolla (2002), [11]Dartnall (1975), [12]Steel (1985), [13]Gruber (2003). Personal communication.

waters. It was with this in mind that Sillman et al. (1996) speculated that the brown smoothhound shark, with a 496 nm rod pigment and much longer rods, might at times frequent somewhat deeper water than the leopard shark.

At first glance, Table 3 would indicate that deep-living sharks are well adapted to their photic environment whereas coastal species are not. However, one must recognize that photons are scarce at great depth and simply catching them must be the overwhelming priority. Coastal species, however, live in a relatively photon rich environment and, moreover, experience major changes in irradiance and spectral composition as they change their distance from the water surface. The visual pigment absorbance of coastal sharks, therefore, probably reflects a compromise between sensitivity and other important concerns such as contrast enhancement (Dartnall 1975).

A Shift in the Visual Pigment Chromophore

In contrast to the sturgeons, sharks have proven more interesting with respect to the lability of the visual pigment chromophore. Until 1990 it was believed that all sharks, indeed all elasmobranchs, possessed visual pigments based only on the vitamin A_1 chromophore, as would be expected of fishes that, for the most part, spend their entire lives in a marine environment. Then, Cohen et al. (1990) analyzed extracts of visual pigment from the rod photoreceptors of six juvenile lemon sharks and a 14 year old adult. Surprisingly, the pigment extracted from the juveniles had a λ_{max} at 522 nm and was based on the vitamin A_2 chromophore, whereas that extracted from the adult absorbed maximally at 501 nm and was based on vitamin A_1 (Fig. 9). Clearly, at some point in the course of maturation the lemon shark switches from one visual
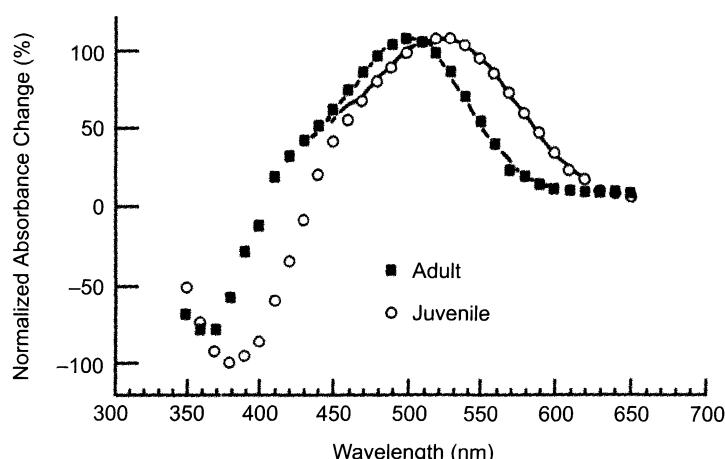
Fig. 9 Normalized difference spectra obtained by spectrophotometric analysis of rod visual pigments extracted from juvenile (open circles) and adult (filled squares) lemon sharks. The line through the open circles is a nomogram for a vitamin A_2-based visual pigment with λ_{max} at 522 nm; that through the filled squares is a nomogram for a vitamin A_1-based visual pigment with λ_{max} at 501 nm. From Cohen et al. (1990).

pigment to the other. This switch appears to be correlated with an age-related change in the habitat and, consequently, the spectral environment, of the lemon shark. The juveniles used in the study were collected from Florida Bay in the Florida Keys, an environment typical of inshore nursery grounds for lemon shark young. These relatively shallow waters are often turbid and are typically milky or yellowish green in color. In contrast, the adult lemon shark inhabits the deeper oceanic waters which are much more blue. It would be advantageous for the visual pigments of the juvenile lemon shark to be 'green shifted' as compared to those of the adult, and it is certainly reasonable to conclude that the visual pigment system of the lemon shark has adapted to the spectral environment .

This conclusion is particularly compelling in view of the fact that a switch from a juvenile vitamin A_2-based system to an adult vitamin A_1-based system is not a characteristic of all shark species. Stimulated by the work of Cohen et al. (1990), Sillman et al. (1996) compared the visual pigments of juveniles and adults of the brown smoothhound shark and the leopard shark. Although there was a small, but significant, difference in peak absorbance of the rod pigment between the two species, there was no difference between juveniles and adults of the same species. Thus, unlike the case with the lemon shark, there is no evidence for a chromophore shift in these two shark species. The failure to develop a labile A_1/A_2 chromophore is probably due to the fact that juvenile and adult leopard and brown smoothhound sharks appear to inhabit the same water along the northern California coast and, therefore, there would seem to be no adaptive value to a shift in chromophore type.

Acknowledgements

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<div style="text-align:right">**3**</div>

Chemosensory Systems in Fish: Structural, Functional and Ecological Aspects

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ABSTRACT

In fish, the chemical senses are represented at least by three separate modalities: the sense of olfaction, the system of solitary chemosensory cells, and the taste system. All three modalities are phylogenetically old and well conserved to this date. The chemical senses enable the fish to orientate, to follow and find food or prey. They help to avoid enemies or predators, but also to interact with conspecifics and to find sexual partners. Chemical senses are common to all systematic groups of fish and are also present in cyclostomes (hagfish and lampreys). As a rule, the chemical senses are of great importance to aquatic vertebrates, and are especially well developed in some night-active species and those living in the dark (e.g. in caves) or in muddy waters. All three chemical senses develop early during ontogeny, and often are functional at time of spawning.

Olfaction is a "distance" sense that enables the fish to locate and find the food or the sexual partner or to avoid an enemy on a greater distance. In contrast, taste serves to detect and approve of the foodstuff that is near to the head or even taken up into the mouth or oropharyngeal cavity. The function of the solitary chemosensory cells is not yet well understood, but these cells may also serve to locate food and/or a nearby predator or conspecific.

The chemical senses of fish were reviewed for several times. They are well represented in handbooks (Beidler 1971a/b; Doty 2003) and monographs, as e.g., edited by Hara (1982, 1992); Farbman (1992), Simon and Roper (1993), Finger et al. (2000). Further, two congress report series exist: "Olfaction and Taste" (since 1962, triannual), and "Chemical Signals in Vertebrates" (since 1976, triannual). A special journal, "Chemical Senses", covers all aspects of the chemosensory systems since 1976.

Key words: Olfaction, Taste, Solitary chemosensory cells, Ultrastructure, Toxic substances

THE OLFACTORY SYSTEM IN FISH

Introduction

The olfactory system of fish and other vertebrates consists of the peripheral part of the olfactory organ proper including the olfactory nerve formed by the axons of the olfactory receptor

neurons (ORNs) and the central part comprising the olfactory bulbs and the higher brain areas involved in processing of olfactory information. The peripheral olfactory system is capable of detecting thousands of odor stimuli that are utilized for food search, intra- and interspecific interaction, orientation, and reproduction. Thus, the olfactory system plays an important role and damage to this system may impair the health of fish and even jeopardize their survival.

Gross Morphology of the Peripheral Olfactory Organ in Fish

The peripheral olfactory organ of fish shows considerable variation depending on systematic groups and ecological habitats. Ample literature is available for all aspects of the olfactory system in fish (e.g. Kleerekoper 1969; Hara 1982, 1992; Yamamoto 1982; Zeiske et al. 1992; Finger et al. 2000). The following description of the olfactory organ is mainly based on the situation in teleosts with only occasional reference to other groups of fish.

Cyclostomes (hagfish and lampreys) are monorhinic: a single median olfactory organ is located in the anterodorsal part of the head. Elasmobranchs have paired olfactory organs that usually sit on the ventral side of the head. In most teleosts the paired olfactory organs are located on the dorsal side of the head (for further details see Zeiske et al. (1992) (Fig. 1a). The peripheral olfactory organ consists of a nasal cavity (olfactory chamber or pit) lined with epithelial cells and ciliated nonsensory cells. An incurrent nostril allows water carrying odorants to enter the cavity. The water flows over the olfactory organ proper and leaves the cavity via an excurrent nostril (Fig. 1b). As an exception, in some teleosts the olfactory organ does not sit in a cavity but is open to the exterior. The water flow through the nasal cavity is created in different ways. It can be driven by the pressure differential between the incurrent and the excurrent nostril due to the forward motion of the fish, and/or propelled by motile kinocilia. Some fish have accessory nasal sacs. These sacs are compressed or expanded by transmission of respiratory pressure changes in the oral cavity or by muscular movements. The sensory olfactory epithelium (OE) is located on the floor of this cavity. The shape of the OE varies greatly. It may be flat or folded into lamellae to increase the surface area. The most frequent shape is a rosette with lamellae radiating from a midline raphe (Fig. 1c). The sensory epithelium is located on both sides of each lamella. The lamellae may have secondary folds (e.g. in salmonids). The number of lamellae varies from a few to many. About 230 lamellae were reported for one olfactory organ of *Hoplopagrus guentheri* (Perciformes) by Pfeiffer (1964). The sensory olfactory epithelium covers both sides of the lamellae separated by the lamina propria, a thin sheet of connective tissue containing blood vessels, nerve fibers, and in some cases melanophores. Distribution of the sensory epithelium varies in different species. Four general patterns have been identified (Yamamoto 1982): a) one continuous sensory area on each lamella bordered by the nonsensory epithelium (e.g. in the zebrafish and the channel catfish, *Ictalurus punctatus*, Fig. 1d, Hansen and Zeiske 1998), b) sensory areas regularly separated by secondary folds (e.g. in the char, *Salvelinus spp.*, Yamamoto and Ueda 1977), c) sensory islets scattered in the nonsensory epithelium(e.g. in the catfish, *Plotosus lineatus*, Theisen et al. 1991), or d) nonsensory islets scattered in an otherwise continuous sensory epithelium (e.g. in the goldfish, *Carassius auratus*, Fig. 1e, Hansen et al. 1999).
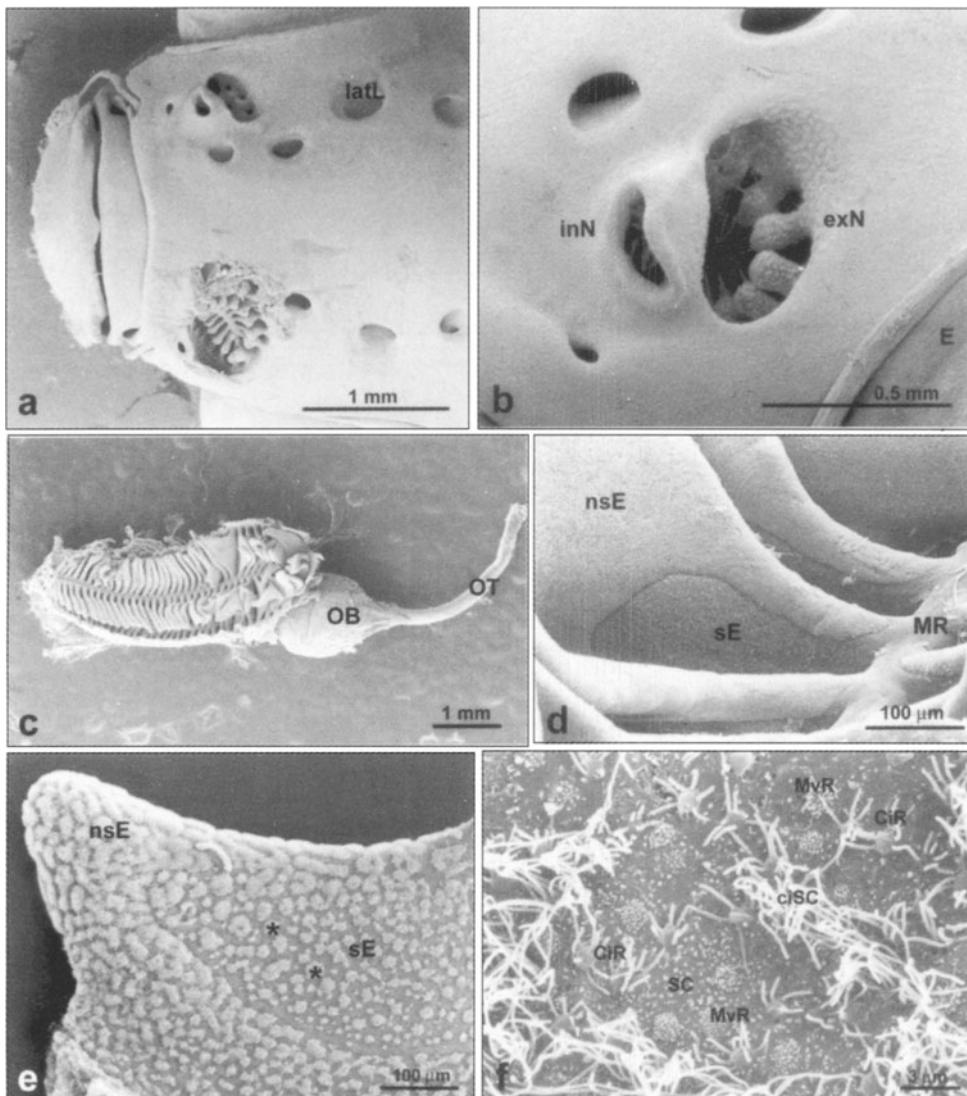
Fig. 1 (a-f) Scanning electron micrographs of the olfactory system of fish.

(a) Zebrafish, *Danio rerio*. Overview of the head. The skin above the left olfactory organ was removed to show the olfactory rosette. latL – opening of the cephalic lateral line system. Scale bar – 1 mm. (b) Zebrafish, *Danio rerio*. A small flap of skin divides the incurrent nostril (inN) from the excurrent nostril (exN). E – eye. Scale bar – 0.5 mm. (c) Channel catfish, *Ictalurus punctatus*. The olfactory rosette, olfactory bulb (OB), and the olfactory tract (OT) were dissected from the head. Note the high number of lamellae radiating from the midline raphe compared to the rosette of the zebrafish in Fig. 1a. Scale bar – 1 mm. (d) Channel catfish, *Ictalurus punctatus*. Apical portion of the rosette. The nonsensory epithelium (nSE) is densely covered by ciliated nonsensory cells. The sensory epithelium (sE) is continuous. MR – midline raphe. Scale bar – 100 μm. (e) Goldfish, *Carassius auratus*. The lamella is divided into nonsensory (nsE) and sensory (sE) epithelium as in the catfish (Fig. 1d), but within the sensory epithelium small islets of nonsensory cells occur (asterisks). Scale bar – 100 μm. (f) Char, *Salvelinus alpinus*. The apical olfactory knobs of ORNs are visible on the surface of the olfactory epithelium: ciliated ORNs (CiR) and microvillous ORNs (MvR). Crypt ORNs are not visible since they do not protrude above the surface of the epithelium. Supporting cells (SC) have small protrusions; ciliated supporting cells (ciSC) have longer cilia than the ciliated ORNs. Scale bar – 3 μm.

Cellular Composition of the Olfactory Epithelium in Fish

The olfactory neurons (ORNs) are bipolar primary neurons and therefore primary sensory cells located in the pseudostratified olfactory epithelium (OE). The OE comprises three principal components: ORNs, nonsensory cells (supporting cells and nonsensory ciliated cells), and basal cells, which are mitotically active and develop into ORNs. ORNs are bipolar with apical dendrites and axons that pass through the basal lamina to project to the olfactory bulb. In the OB, the axons synapse onto mitral cells and local interneurons forming spherical neuropil structures, the glomeruli. Glomeruli are histologically distinct units that serve as basic modules in information processing (Shepherd 1994) and as a relay station to several higher brain areas (Satou 1992).

Three types of ORNs have been identified in actinopterygian (ray-finned) fishes: ciliated, microvillous (for review see Yamamoto 1982; Zeiske et al. 1992), and crypt ORNs. Ciliated and microvillous ORNs are spindle-shaped and their dendrites form an olfactory knob at the surface of the OE (Fig. 1f). From these olfactory knobs arise either some specialized, non-motile cilia (approx. 4-10, Fig. 2a) or many small microvilli (usually 30-80, Fig. 2a). In some fish species, the knobs of ciliated ORNs also bear microvilli (Fig. 2b). The third type of ORNs also bears cilia and microvilli. However, the morphology of this cell type differs greatly from that of ciliated ORNs. Crypt ORNs are egg-shaped and do not extend olfactory knobs (Hansen et al. 1997). They are characterized by submerged cilia in the upper portion of the cell. These cilia do not extend above the surface of the OE. The apical rim of the crypt ORN is equipped with microvilli (Fig. 2c). The cilia may or may not have inconspicuous rootlets according to the species examined. The lower portion of crypt ORNs is abundant in polyribosomes. The upper portion around the crypt mostly contains mitochondria and a fine matrix of cytoplasm. Although crypt ORNs occur regularly in all lamellae, the absolute number of this cell type is low. Due to this fact this cell type has been overlooked till recently. However, a study on the olfactory system of both marine and fresh-water actinopterygians proved that this cell type is widely distributed in bony fishes (Hansen and Finger 2000). The ORNs share several cytological features. ORNs are rich in polyribosomes, rough endoplasmic reticulum and mitochondria. The nuclei of all three types show a typical "checker-board" pattern of chromatin. Nuclei of the ORNs are situated in one or a few layers above the layer of nuclei of the supporting cells. The dendrites of ciliated and microvillous ORNs contain longitudinally orientated microtubules and long mitochondria. The cilia in ciliated ORNs (about 3 to 10) and crypt ORNs (up to seven) contain an axonemal complex with the typical 9+2 pattern of microtubules (Yamamoto 1982; Hansen and Zeiske 1998). Cyprinodontiform fish and hagfish build an exception. Their pattern of microtubules varies (for review see Zeiske et al. 1992). In some species cilia of ciliated and/or crypt ORNs have rootlets, but they are seldom and less pronounced than in nonsensory ciliated cells (Zeiske et al. 1992; Hansen and Finger 2000). A typical feature of microvillous ORNs are centrioles buried deep in the dendrite. This characteristic led to the assumption that microvillous ORNs are immature pre-stages of ciliated ORNs (Bannister 1965). This assumption has been dismissed since studies on the development and regeneration of the olfactory epithelium proved that ciliated ORNs appear prior to microvillous ORNs (Breucker et al. 1979; Evans et al. 1982; Hansen et al. 1999).
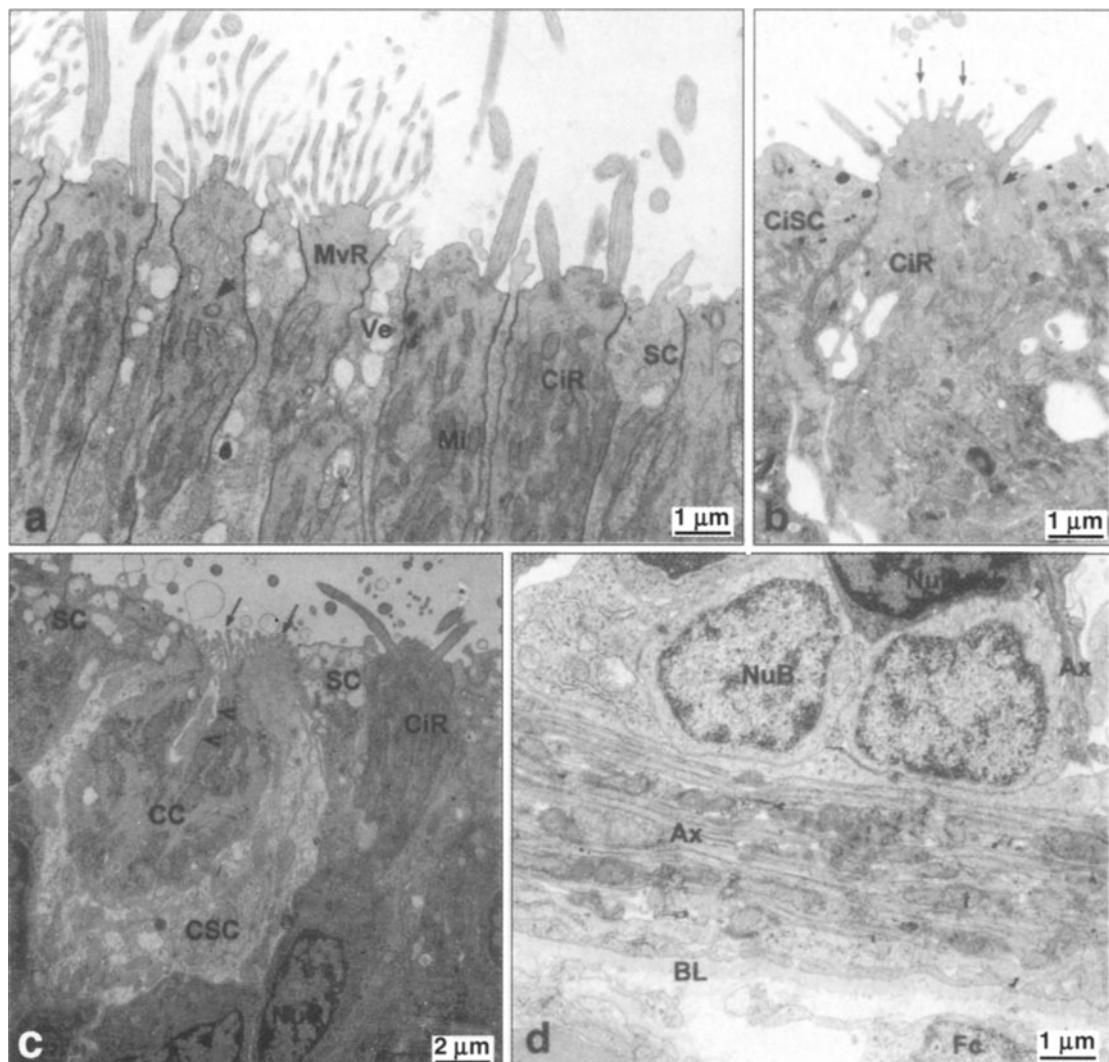
Fig. 2 (a-d) Transmission electron micrographs of the olfactory epithelium of fish.

(a) Goldfish, *Carassius auratus*. Microvillous (MvR) and ciliated (CiR) ORNs. Microvillous ORNs contain the typical centrioles (arrow) in the upper portion of the dendrite. Numerous mitochondria (Mi) are longitudinally arranged in the ORNs. Supporting cells (SC) show vesicles (Ve) and small protrusions. Scale bar – 1 μm. (b) Bichir, *Polypterus senegalus*. In this species ciliated ORNs have also microvilli (arrows). A ciliated supporting cell (CiSC) shows the cross section of the basal part of a cilia. Ciliated supporting cells contain small electron dense vesicles. Scale bar – 1 μm. (c) Zebrafish, *Danio rerio*. Section through the upper portion of a crypt ORN (CC) with the typical insunk cilia (arrowheads). Crypt ORNs are surrounded by specialized supporting cells (CSC). Note that both the crypt ORN and the specialized supporting cell(s) have microvilli at their apical ends (arrows). NuR – nucleus of an ORN showing the typical "checkerboard pattern" of chromatin. SC – supporting cell. Scale bar – 2 μm. (d) Zebrafish, *Danio rerio*. Basal region of the olfactory epithelium with two basal cells. Their nuclei (NuB) are lighter than the nucleus (NuR) of the adjacent ORN. Axons (Ax) stretch vertically towards the base of the olfactory epithelium where other axons have accumulated between the basal cells and the basal lamina (BL). Fc – fibrocyte in the lamina propria. Scale bar – 1 μm.

Supporting cells are cylindrical epithelial cells extending from the basal lamina to the surface of the OE. The most basal part of these cells maintains foot processes that reach between the adjoining basal cells and the somata of the ORNs. Their apical endings may bear microvilli-like protrusions (e. g. cyprinids) and/or cilia (e. g. salmonids). Bundles of intermediate filaments (tonofilaments) are characteristic features of supporting cells. These bundles are arranged either longitudinally or horizontally. The latter form the so-called terminal web beneath the surface of the OE at the level of the junctional complex. In some species, the upper portion of the supporting cell contains vesicles of various shape and electron density (for review see Yamamoto 1982; Zeiske et al. 1992). Nuclei of supporting cells lack the prominent checkerboard pattern of ORN nuclei.

Apart from their main function as supportive elements, several other functions have been attributed to the supporting cells: they secrete components into the mucus overlaying the OE, electrically isolate ORNs, and contain detoxifying enzymes (Okano and Takagi 1974). Also, they contain P450-like enzymes which are thought to modify and/or inactivate odorants (Lazard et al. 1991).

Ciliated nonsensory cells are also cylindrical cells stretching from the basal lamina to the epithelial surface but they lack basal foot processes. On the flat apical endings of these cells occur kinocilia, their amount ranging from a few to more than 60 cilia per cell. These cilia are equipped with the 9+2 axonemal structure and a complex rootlet system typical for kinocilia. Kinocilia are motile and their skeletal components indicate that these cilia are capable of propelling water and/or mucus (Sleigh 1989).

Cell Turnover in the Olfactory Epithelium of Fish

The OE is unique insofar as olfactory neurons have a limited life span and are substituted continually. Due to the life-long renewal of the OE, basal cells play an important role. These undifferentiated cells are roundish and sit on the basal lamina of the OR. They differentiate to replace dying ORNs during the regular turnover of the OE (Fig. 2d). Extirpation of the OB or olfactory nerve transection leads to complete degeneration of the nervous part of the OE (for review see Zippel (1993). The layer of basal cells, however, is capable of regenerating the OE. Studies aimed at examining the morphological, physiological, and behavioral recovery showed that the OE had reached control levels 8 months after axotomy in goldfish (Zippel et al. 1997b).

The Olfactory Bulb and Central Projections in Fish

Olfactory neurons are primary sensory cells, i.e. their axons project to the olfactory bulb (OB), the first relay station in the brain. The unmyelinated axons accumulate in the lamina propria of the olfactory organ and build the so-called fila olfactoria. The fila olfactoria in turn form the I. cranial nerve, the olfactory nerve. The olfactory nerve varies in length in a species-specific way. In general, fish with short olfactory nerves (e.g. goldfish *Carassius auratus*, channel catfish *Ictalurus punctatus*) have pedunculated OBs, i.e. long olfactory tracts (OTs). The OTs form the connection between the OB and the higher brain centers. Fish

with long olfactory nerves tend to have short OTs, i.e. "sessile" OBs, which are located next to the telencephalon proper (e.g. eel, *Anguilla anguilla*, swordtail *Xiphophorus helleri*). The reasons for the two types of morphology are not well understood. At least for some fish constraints of body size may play a role, since even closely related species show different morphologies. The zebrafish, *Danio rerio*, a cyprinid with a short snout and relatively big olfactory organs has a short olfactory nerve and sessile OBs that sit right next to the telencephalon. The goldfish, *Carassius auratus*, also a cyprinid, has pedunculated OBs with rather long OTs.

Olfactory nerve endings branch at their very end and synapse on the second-order neurons, the mitral cells. The neuropils of the axon's nerve endings and the dendrites of the mitral cells form distinct morphological structures, the glomeruli. The morphology of the OB is rather consistent across the vertebrate lineage. Distinct layers of cells process the incoming information and convey it to the higher brain centers. The layers in fish are from outside to inside: (i) the olfactory nerve layer, (ii) the glomerular layer, (iii) the mitral cell layer, and (iv) the internal cell layer. However, glomeruli and lamination are not quite as prominent as they are in rodents. Glomeruli (about 80 – 130) are sometimes "fuzzy" and not well-defined (Baier et al. 1994; Baier and Korsching 1994). The glomeruli of one OB are arranged in a stereotyped pattern and are bilaterally symmetric to the other OB. Distinct cell types of ORNs project to well-defined areas of the OB as shown by retrograde tracing (Morita and Finger 1998; Hansen et al. 2001).

Fish mitral cells have a large cell body and more than one dendrite. In addition to the synaptic input from ORNs, mitral cells form numerous dendro-dendritic reciprocal synapses with granule cells of the internal cell layer. Another type of neuron occurs between the mitral cells, the ruffed cell (Kosaka 1980; Kosaka and Hama 1981; Zippel et al. 1999). These cells synapse to other neurons and surround mitral cell dendrites resembling glial cell processes (Kosaka 1980). Local interneurons (granule cells) of the internal cell layer receive input from centrifugal fibers from the higher telencephalon (Ichikawa 1976).

Axons of the mitral cells and the ruffed cells run through the OT and convey their information to the telencephalon. The OT is divided into two bundles, the lateral olfactory tract (LOT) and the medial olfactory tract (MOT). Both bundles are divided further into smaller bundle (Sheldon 1912). Each bundle conveys distinct classes of information (Sorensen et al. 1991; Hamdani et al, 2001a; Hamdani et al. 2001b). The fibers of the OT terminate in the medial terminal field in the area ventralis telencephali, the lateral terminal field in the ventrolateral part of the area dorsalis telencephali, and the posterior terminal field in the central part of the area dorsalis telencephali (Oka et al. 1982; Satou 1990; Huesa et al. 2000). For more detailed information and ample references see (Satou 1992).

Processing of Olfactory Information

How the olfactory system distinguishes between vast numbers of odorants and processes this information is an intriguing problem. The initial event takes place at the surface of the epithelium. Odor molecules bind to specific receptor proteins in the membrane of cilia or microvilli of the ORNs and thus trigger a mechanism that transduces the chemical signal into electrical activity.

A large multigene family encoding about 1000 putative olfactory receptor proteins was first discovered in mammals (Buck and Axel 1991). In fish, the receptor repertoire is much smaller with approx. 100 genes (Ngai et al. 1993c; Barth et al. 1996; Cao et al. 1998). The receptor proteins, all of which so far are G-protein-coupled, are expressed in a fraction of ORNs that are scattered in the OE. Contrary to the scenario in rodents, no obvious pattern of distribution was found in catfish (Ngai et al. 1993b; Ngai et al. 1993a). In zebrafish, receptor molecules of one type are expressed in "rings" across the olfactory organ (Weth et al. 1996).

Two different receptor families were identified in goldfish. One gene family (GFA) comprises putative odorant receptors similar to those found in rodents (OR-type) and other fish. Members of the second family (GFB) resemble putative pheromone receptors (V2R-type) found in the vomeronasal organ of rodents and also in pufferfish (Cao et al. 1998). *In situ hybridization* experiments with molecular probes to some of these receptor proteins revealed that in goldfish the OR-type receptor proteins are expressed in ciliated ORNs and the V2R-type receptor proteins are expressed in microvillous ORNs (Hansen et al. 2002).

The interaction of receptor molecules with G-proteins (heterotrimeric GTP-binding proteins) regulates specialized enzymes, which in turn control second messengers. These second messengers target ion channels, which gate an influx of ions across the cell membrane. As a consequence of the change in membrane potential, action potentials are generated in the axon. Gαolf, a member of the Gαs family, mediates transduction via the type III adenylyl cyclase/cAMP pathway. Other transduction pathways mediated by members of the Gαo and the Gαq families and utilizing IP3 (inositol triphosphate) and DAG (diacylglycerol) have been reported for fish (for review see Ache and Restrepo 2000).

Physiology of Olfaction in Fish

Electrophysiological experiments with neural recordings from the surface of the OE, under water electro-olfactogram (EOG) recordings from the water above the OE, electroencephalographic (EEG) recordings from the bulb as well as single cell recordings from bulbar cells and dissociated cells of the OE revealed an amazing sensitivity to odorants. For instance, in the catfish electrophysiological thresholds for the more effective amino acids determined by EOG ranged between 10^{-7} and 10^{-10} M (Caprio 1978, 1982). These results confirmed experimental results from the beginning of the 20th century which described the sense of smell (in contrast to the sense of taste) as a "distance" sense capable of detecting information about minute quantities of food-related or socially-related compounds in the surrounding water (Olmsted 1918; Parker and Sheldon 1919; Sheldon 1919).

Several classes of odorants are biologically relevant in the olfactory system of fish, e.g. amino acids and nucleotides as potent food cues, bile salts, pheromones and compounds of urine for social and reproductive interaction (Sorensen and Caprio 1998).

Various studies with biologically relevant odor stimuli aimed at the specificity of the three morphologically different types of ORNs for the different classes of odorants.

Functional, anatomical, biochemical, and molecular studies revealed that the three types of ORNs, indeed, respond to different odorant classes. Ciliated ORNs mediate responses to bile salts (Thommesen 1983; Hansen et al. 2002a) and microvillous ORNs mediate responses to nucleotides (Hansen et al. 2002a). Amino acids are detected by both microvillous and ciliated ORNs (Sato and Suzuki 2001; Speca et al. 1999; Hansen et al. 2002a), and pheromones may be detected by a subset of microvillous and/or crypt ORNs (Zippel et al. 1997a; Sorensen et al. 2002). In rainbow trout, ciliated ORNs are considered "generalists" that respond to a wider variety of odorants, whereas microvillous ORNs are "specialists" that respond to more specific compounds (Sato and Suzuki 2001).

Environmental Aspects

Located at the interface of the environment and the nervous system, the peripheral olfactory organ is susceptible to damage by xenobiotics. In the nasal cavity, the dendrites of the olfactory neurons are in direct contact with the external world and, thus, exposed to toxic substances produced by industrial and agricultural activities. As primary neurons, ORNs are also in close contact with the central nervous system by their axonal projections to the olfactory bulb. The olfactory pathway has also been suggested as an anatomical route for toxic agents into the brain (Hastings and Evans 1991).

Among water pollutants, copper ions and mercury salts have been thoroughly studied. Exposing fish to mercurial compounds leads to deviant behavioral reactions and suppressed electrical responses in the olfactory bulb (Hara et al. 1976) and to impaired EOG responses of the OE (Baatrup et al. 1990). Also, the toxicity of copper upon the olfactory system of fish has been amply studied. Exposure to copper ions causes degeneration of ORNs (Julliard et al. 1993; Moran et al. 1992) and results in the reduced ability to discriminate odorants (Saucier et al. 1991). Pesticides, herbicides, insecticides, and fungicides have created a serious problem of pollution and environmental toxicity. Fish perceiving these substances with their olfactory system try to escape to safer areas (Giattina and Garton 1983; Ishida and Kobayashi 1995). The damaging effects of e.g. dichlobenil, a potent herbicide, on the olfactory system has been shown (Andreini et al. 1997; Ishida et al. 1996), for a detailed review see Klaprat et al. (1992). An important aspect of olfactory-mediated behavior that can be impaired by water pollution is the homing of fish. Homing, i.e. the ability to find their way back to a "home", usually a spawning area, has been well-documented for migratory and non-migratory fishes (Gibson 1993). The Pacific salmon (*Oncorhynchus* spp.) wanders thousands of miles through the Pacific Ocean before returning after years to its home stream with an amazing accuracy. This accuracy is due to the salmon's ability to recognize the odor of the stream where it had been spawned (Hasler and Scholtz 1983, for detailed information see Nevitt and Dittman this volume). Consequently, damage to the olfactory system has a severe impact on health or even survival of fishes.

One way of dealing with this problem is the life-long turnover of ORNs. ORNs have a limited lifespan and the mitotically active basal cells continuously replace dying neurons. Consequently, ORNs that may have been damaged by xenobiotics are removed on a regular basis.

Another defense mechanism to protect the fragile OE is the availability of detoxifying enzymes with activities similar to those observed in the liver (Dahl and Hadley 1991). The tripeptide glutathione (γ-glytamylcysteinylglycine, reduced GSH) and the GSH conjugating enzyme glutathione S-transferase (GST) protect cells from damage by free radicals (also produced during degenerative processes during ORN turnover), xenobiotics, electrophiles, and peroxides (Starcevic and Zielinski 1997a; Starcevic and Zielinski 1997b). However, these mechanisms can only shield the OE to a certain extent and extensive and/or long-lasting pollution of our rivers, lakes, and oceans will doubtlessly harm fishes as well as other creatures.

THE SYSTEM OF SOLITARY CHEMOSENSORY CELLS IN FISH

Introduction

In addition to the olfactory and the gustatory system, fish (and ranid tadpoles) possess another sensory system that is chemoreceptive, the solitary chemosensory cells (SCCs). Due to the variability in their morphology and distribution and the resulting difficulties with respect to physiological experiments, their neuronal connection, let alone their function is poorly understood except for few specialized teleosts (see below).

In the 19th century, Kölliker (1886) found spindle-shaped, presumed sensory cells (Stiftchenzellen) in the skin of tadpoles. Morrill (1895) described such cells for the epidermis of the free pectoral fin rays of sea robins. Ultrastructural investigations of SCCs showed that these cells morphologically resemble taste receptor cells and that they are associated with nerve fibers (Whitear 1965). SCCs were found in a variety of fish groups from sturgeons to percomorphs and also in lampreys and lungfish (for review see Whitear 1992). Braun and Northcutt (1998) studied the so-called "Schreiner organs" in hagfish and concluded that in hagfish SCCs agglomerate and form a unique organ that is not homologous to taste buds in vertebrates.

Distribution of Solitary Chemosensory Cells

SCCs comprise a diffuse system of bipolar secondary sensory cells. The skin of most fish is not keratinized but covered with mucus. SCCs are embedded in the epidermis and dispersed as isolated cells across the outer body surface. They also occur in the epithelia of the oropharyngeal cavity, the gills, and even in the olfactory epithelium of goldfish (Hansen and Zippel 1995; Hansen et al. 1999). Although SCCs are widespread, their quantity varies immensely. Also, they are not evenly distributed over the fish's body. The largest number of SCCs has been found on the fin rays of the anterior dorsal fin of rocklings. This fin is modified by reduction of the skin web between the rays and increase of rays and serves as a special sensory organ (see below). Kotrschal and Adam (1984) counted SCC densities of up to 1.0×10^5 per mm^2 in *Gaidropsarus mediterraneus*, and although the SCCs occur at much lower densities elsewhere in the skin, it has been estimated that a rockling of 20 cm total length carries between 3 to 6 million SCCs. Total numbers of SCCs found in other fish groups are

much lower, yet the total number of SCCs per fish seems to be much higher than the total number of taste bud cells (Kotrschal 1991). In cyprinids densities vary between 2000 and 4000 SCCs per mm^2. Two species of catfish revealed densities of 1000 to 2000 SCCs per mm^2. The lowest densities counted were found in the neon tetra, *Hyphessobrycon innesi* with 250 SCCs per mm^2 (Kotrschal 1992). In some fish, SCCs are either absent or so poorly developed that they escaped detection (e.g. in the stickleback, *Spinachia spinachia* and in the mud skipper, *Periophthalmus koelreuteri* (Whitear 1992). During ontogeny, SCCs occur prior to taste bud cells. In the zebrafish, *Danio rerio* their numbers increase sharply after hatching to about 25 days after fertilization and remain relatively constant thereafter (Kotrschal et al. 1997). Interestingly, cells that strongly resemble SCCs have recently been reported for mammals in developing vallate papilla of rats (Sbarbati et al. 1998) and in the respiratory part of the nasal cavity of adult mice and rats (Böttger et al. 2001).

Cytology

In general, SCCs are spindle-shaped cells, however, there is some variation which often corresponds to the variation in gustatory cell cytology in different species (Whitear 1992). In thinner epithelia, the shape of the SCC may be roundish or inclined to one side. The apical ending of the SCC extends above the surface of the epithelium either as one stout projection of 1 – 2 μm (Fig. 3a,c) or as several smaller microvilli-like structures that often sit on a common base (Kotrschal 1991). In the zebrafish, SCCs in embryos and early larvae have an apex divided into several microvilli (Fig. 3b). In adult zebrafish, the majority of SCCs possess only one stout villus. It is not clear whether the branched type/single villus type represent developmental stages of the same cell or whether the apices change during the life stages of the fish by a succession of new cells (Kotrschal et al. 1997).

In other fish groups, the SCCs show divided apices as the general type, as e.g. in clupeids (Fox et al. 1980) and lampreys (oligovillous cells, Whitear and Lane 1983) as well as in the olfactory epithelium of goldfish (Hansen et al. 1999).

The cell body of SCCs contains many mitochondria, a Golgi system, and longitudinally arranged microtubules sometimes associated with microfilaments. Vesicles are usually abundant and occasionally also occur in the apical villus. Size and electron density of the vesicles vary in different groups of fish (50 – 70 nm in diameter in cyprinids and silurids). Vesicles of different size and electron density may even occur in one cell. The nucleus is usually embayed or lobulated (Whitear 1992).

Innervation

SCCs are secondary sensory cells. Slender nerve fibers contact the SCC mostly near the base of the cell. These nerve fibers occasionally indent the cell body so that they are almost wrapped by the SCC. Synaptic specializations are inconspicuous and resemble gustatory synapses: pre-and postsynaptic densities are fuzzy. Occasionally small vesicles are seen on the presynaptic side. The nerve fibers contacting the SCCs belong to different nerves (cranial or
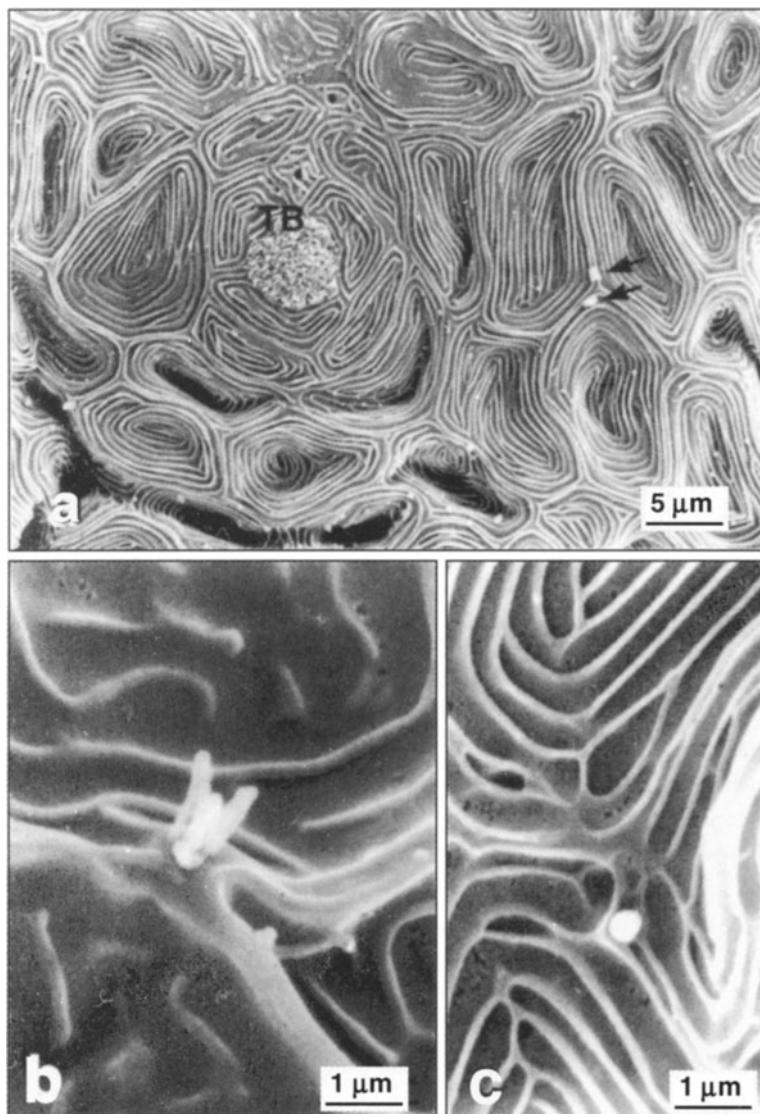
Fig. 3 (a-c) Scanning electron micrographs of solitary chemosensory cells of fish.

(a) Black convict, *Archocentrus nigrofasciatus*. Taste bud in the skin of the head and two SCCs with one stout villus each (arrows). The skin shows the microridges typical for fish skin. Scale bar – 5 µm. (b) Zebrafish, *Danio rerio*. Larva 8 days after fertilization. The apical ending of the SCC is divided into several small villi. Scale bar – 1 µm. (c) Goldfish, *Carassius auratus*. SCC with one stout apical villus in the skin of the head. Scale bar – 1 µm.

spinal) depending on the location of the SCC (Whitear 1952; Whitear and Kotrschal 1988; Kotrschal and Finger 1996; Kotrschal et al. 1998). SCCs on the body of teleosts are most probably innervated by branches of the spinal nerves although this has not yet been proved experimentally. In the catfish, *Ictalurus melas*, the recurrent branch of the facial nerve (the

VII. cranial nerve) was cut. As a result the taste buds on the flank degenerated but the SCCs did not disappear (Lane 1977 as cited in Whitear 1992). This could either mean that the SCCs are not trophically dependent on the recurrent nerve or that the SCCs are innervated by spinal nerves.

Two groups of teleosts with a highly specialized SCC system were thoroughly investigated: rocklings and sea robins. As mentioned before, rocklings posses a specialized vibratile anterior dorsal fin. It is well suited for studying SCCs since it is equipped with millions of SCC but no taste buds. The fin is innervated by fibers of the dorsal recurrent branch of the facial nerve despite the presence of spinal nerve fibers in that area (Whitear and Kotrschal 1988; Kotrschal et al. 1993). Sea robins possess specialized pectoral fins. The SCCs in these fins are innervated by spinal nerves (Finger 1982). For more details see below.

Physiology of the SCC System in Fish

Electrophysiological experiments are complicated if not impossible due to the scattered distribution of SCCs across the body and the presence of taste buds in the same areas. Thus, investigations are limited to few groups of fish with SCC specializations. Lampreys have plenty of SCCs (oligovillous cells) but no taste buds. Baatrup and Døving (1985) provided electrophysiological evidence that SCCs in the lamprey are chemosensory cells. Oligovillous cells of lampreys responded to acetic acid, NaCl, sialic acid, mucoid substances and thawwater from trout. Recordings were done in areas where no Merkel cells (associated with tactile nerve fibers) were present. Peters and van Steenderen (1987) recorded from the recurrent branch of the facial nerve of rocklings (*Ciliata* and *Gaidropsarus*). Responses were evoked to urine of other rocklings, water in which rocklings were kept and diluted human saliva. Responses to typical chemical stimuli like amino acids, salts, and acids were weak or absent. Further studies suggested that the SCCs of the vibratile fin is used to test the environment for predators (for references see (Whitear 1992). Interestingly, the specialized SCCs system in rocklings is centrally connected like the gustatory system although it is behaviorally used for predator avoidance, not feeding (Kotrschal et al. 1993; Kotrschal and Finger 1996; Finger 1997a).

The third specialized SCC system without interference of taste buds is found in sea robins as mentioned above. These benthic fish probe the substrate for food by means of their specialized pectoral fins (for further references see Kotrschal (1991). The innervation of these SCCs is exclusively spinal. Electrophysiological experiments showed that *Prionotus carolinus* detects several amino acids at concentrations from 10^{-6} to 10^{-4}M (Silver and Finger 1984). The types of amino acids detected are similar to those detected by the gustatory system of some other fish, especially marine species (Kiyohara and Hidaka 1991). Centrally the SCC system on the pectoral fins of *Prionotus* is connected like a somatosensory system although it is used behaviorally in food search similar to the way other fish use their taste system (Finger 1997a, 2000).

Biochemical information about the system of SCCs is rare. Arginine is a potent taste stimulus in fish (Caprio et al. 1993). In catfish, an arginine receptor was described that reacts

with the lectin PHA-E (*Phaseolus vulgaris* erythroagglutinin) and an antibody specific for this receptor (Grosvenor et al. 1996). The lectin as well as the antibody labels some taste bud cells in catfish and additionally shows arginine receptor-like binding in the SCCs (Finger et al. 1996). Thus, SCCs and some taste bud cells seem to share a common feature, the putative arginine receptor (Finger 1997a).

To our knowledge no study dealing with environmental influences on SCCs is available. However, since the chemosensory receptor cells of the SCC system are in direct contact with the surrounding world, it is highly likely that the SCCs are affected by toxic substances in a similar way as the systems of olfaction and taste.

THE TASTE SYSTEM IN FISH

Introduction

As in other vertebrates, the taste system in fish comprises two parts, the peripheral and the central taste system. The peripheral part includes the taste organs, the so called taste buds (TBs) and their afferent (and efferent) nerves, whereas the central part consists of the nuclei of these nerves in the oblongate medulla and some other nuclei in higher brain areas.

The peripheral and, to some extent, the central taste system of fish were often reviewed. The older work is comprised in reviews by Kolmer (1927) and Boeke (1934), and the more recent studies by Cordier (1964); Bardach and Atema (1971); Murray (1971); Kapoor et al. (1975); Reutter (1978, 1982, 1986, 1992); Jakubowski and Whitear (1990); Witt (1996); Reutter and Witt (1993); Sorensen and Caprio (1998); Finger and Simon (2000); Jakubowski and Żuwala (2000); Tagliafierro and Zaccone (2001); Witt et al. (2003).

Distribution of Taste Buds in Fish

TBs are intraepithelial or intraepidermal organs and occur within the epithelia of the mouth and headgut and in the branchial region (internal TBs); they also may be found on the surface skin of the fish's head and body and especially on appendices of the body, like the barbels, fins and solitary fin rays (external TBs). TBs are especially numerous in scale-less fishes that are adapted to the dark, as most of the catfishes. For instance, in the bullhead, *Amiurus*, 25 TBs are situated in one square millimeter of barbel skin; the skin of the head contains up to 7 TBs per mm^2, and the skin of the dorsal trunk about 9 TBs per mm^2. Altogether, a 25 cm long bullhead has about 175 000 external (90%) and about 20 000 (10%) internal TBs; a total of about 200 000 TBs (Atema 1971). The number of TBs is greatly dependent on the fish's size: a 5 cm long channel catfish, *Ictalurus melas*, has a total of about 11 000 TBs, whereas a 40 cm long fish has roughly 680 000 TBs (Finger et al. 1991). In a 6 cm long minnow, *Pseudorasbora parva*, the relation of external to internal TBs is 1500 to 6 600, with densities of TBs up to 140 per mm^2 at inner lips and the palatal organ (Kiyohara et al. 1980). In the palatal organ of the brook char, *Salvelinus fontinalis*, 40 TBs occur per mm^2 (Hara et al. 1993), and in the same organ, a maximum of 820 TBs per mm^2 were counted in the
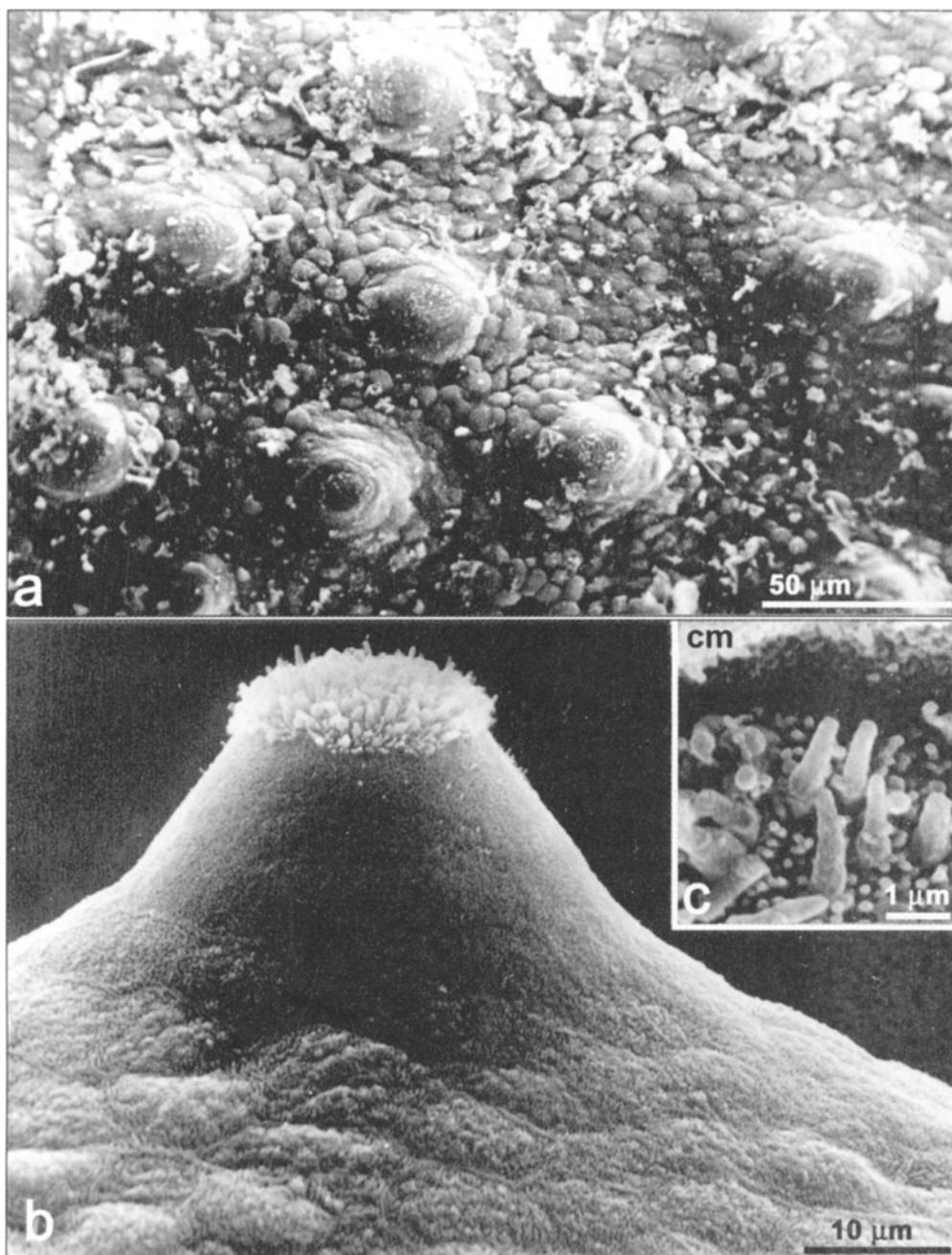
Fig. 4 (a-c): Scanning electron micrographs of *Amiurus (Ictalurus)* taste buds. a): Part of a maxillary barbel with several slightly elevated taste bud-containing epidermal hillocks. b): Lateral view of an epidermal taste bud-containing hillock (type III taste bud) with the apically situated receptor area. c): Almost vertical view of the lateral part of the receptor area that consists of large and small receptor villi. Cm - marginal cell. Scale bars: a) 50 μm, b) 10 μm, c) 1 μm.

carp, *Cyprinus carpio* (Osse et al. 1997)! To summarize, numbers of TBs and their main localities in fish vary and are species specific.

Morphology of Fish Taste Buds

TBs are elongated, pear-shaped or oval organs, about 80 – 100 μm high and about 40 – 60 μm wide. With their broader bases they may "sit" atop of dermal papillae, their bodies lie entirely within the epithelium and their narrow apical parts at the epithelium's surface (refs. as cited above). TBs may be situated in epithelial hillocks and then are markedly elevated above the average surface level of the epithelium; they also can be less elevated or may end apically at the epithelial surface (TB types I, II and III; Reutter 1973, Reutter et al. 1974). As a rule, the more elevated TBs sit on the more exposed parts of the fish's body, as for instance, the lips, the breathing valves, the gill rakers, and the barbels (Fig. 4a,b). Possibly these type I TBs are deflected by food particles and may also be mechanosensitive (Reutter 1971, 1978).

Cell Types and Cellular Specializations in Fish Taste Buds

The following description of TB ultrastructure is the essence of numerous investigations that were reviewed for several times (see citations above). For this reason, citations are given only occasionally and for more recent results.

A TB comprises several different kinds of cells: the main part of the bud consists of its sensory epithelium that contains two main cell types of different electron density (by using transmission electron microscopy, TEM), the light cells and the dark cells. These slender cells run parallel to the TB's longitudinal axis and terminate apically with microvillar processes, which together form the TB's receptor area (Fig. 4b,c). The nuclei of these slender cells are located in the lower third of the sensory epithelium, the widest part of the TB. One to 5 basal cells lie at the base of the TB. These flat, disc-shaped cells are oriented transversely to the bud's longitudinal axis and fill slight depressions of the basal lamina, which is situated between the TB (and the normal epithelium) and the connective tissue of the dermal papilla bearing the TB. The dermal papilla contains a blood capillary and the TB's nerve. This nerve lacks myelin sheets where it penetrates the basal lamina. The then unmyelinated axons occupy the region between the basal processes of the elongated cells and the basal cells and intermingle with them. Here, in the so-called nerve fiber plexus of the TB, synapses occur. Marginal cells lie at the border between these main structures of a TB and the surrounding non-specialized epithelium. These cells not only form the interface between the TB-bearing epithelium and the TB proper but also are involved in the TB's cell turnover (see below).

Regarding the nomenclature, the cells comprising the TB's sensory epithelium are named and interpreted controversially. The electron lucent light cells are also named gustatory cells or sensory cells and the dark cells sustentacular or supporting cells (Whitear 1971; Jakubowski and Whitear 1990; Jakubowski and Żuwala 2000; Tagliafierro and Zaccone 2001). But as previously discussed (Reutter 1978, 1982; Reutter and Witt 1993; Witt et al.

2003), there is some evidence that also the dark cells are synaptically connected to the (dendritic) axons of the TBs nerve fiber plexus indicating also a sensory function. It seems likely that the morphology of dark cells, especially their richness in intermediate filaments and their lobar processes ensheathing the more roundish light cells is not sufficient to define them as pure sustentacular cells. Therefore, we prefer the following definition: The TB sensory epithelium consists of light cells and dark cells (**not** light sensory and dark sensory cells!), basal cells and the nerve fiber plexus.

The **light cells** are spindle-shaped and roundish in cross section. Their nuclei are round- ish or oval and only faintly lobed. The cytoplasm is rich in organelles and often, particularly in the cell's lobed basal processes, rich in synaptic vesicles (see below). This cell is rich in tubular profiles of smooth endoplasmic reticulum especially in the supranuclear region. Inter- mediate filaments are scarce. Microtubules are running parallel to the cell's length. The apex of the cell is mostly formed by one large microvillus, 1.5 μm long and about 0.3 μm in diameter. Its cytoplasm contains longitudinally arranged microfilaments, a few microtubules and, at its base, some tubular profiles of endoplasmic reticulum (Figs. 5 a-d).

The **dark cells** are also slender and, especially in the upper two thirds of the bud, star- shaped in cross section: they possess sheet-like lateral processes by which they surround the light cells. The plasmalemmata of both cells interdigitate and are fixed by desmosomes. The nuclei are elongated or oval and often lobed. The cells bases are also divided into several slender processes that may contain synaptic vesicles. Intermediate filaments are rich throughout the cytoplasm, especially in the basal processes and in the supranuclear region. Microtubules are rare. Rough endoplasmic reticulum and large vesicles (containing components of the later surface mucus) are regularly found. Dark cells terminate apically with several small microvilli of about 0.5 μm length and about 0.2 μm width. They also contain longitudinally arranged microfilaments (Figs. 5 a-d).

Cells of intermediate electron density, also rich in organelles, occur besides the light and the dark cells. These cells seem to be not yet differentiated (see below). Further, a TB also contains cells with signs of apoptosis, or even the detritus of dead cells.

The **receptor area** of a TB consists of the apical microvillar endings of the sensory epithelium's elongated cells. As seen especially in the SEM, the receptor area can protrude the epithelial hillock which contains a TB (type I TBs, Fig. 4 b); it might be also slightly sunken in especially in type III TBs. In this case, the apical marginal cells are arranged in a ring-like manner resembling the taste pore of a mammalian TB (Fig. 4c). The diameter of a receptor area varies between 5-15 μm. In numerous species of fish, the receptor area contains the large recep- tor villi (which belong to the light cells) and the small receptor villi (the apical endings of the dark cells) (Figs. 4c, 5a).

The **basal cells** are relatively electron lucent cells with large nuclei. They also are rich in organelles and contain, especially near the nerve fiber plexus, numerous synaptic vesicles (Figs. 6, 7c). In some species the basal cells protrude with spine-like microvillar processes into the neighboring nerve fiber plexus (*Amiurus*: Desgranges 1966; Royer and Kinnamon 1996); *Silurus*: Reutter 1986, 1987; Jakubowski 1983; *Astyanax*: Boudriot and Reutter 2001).
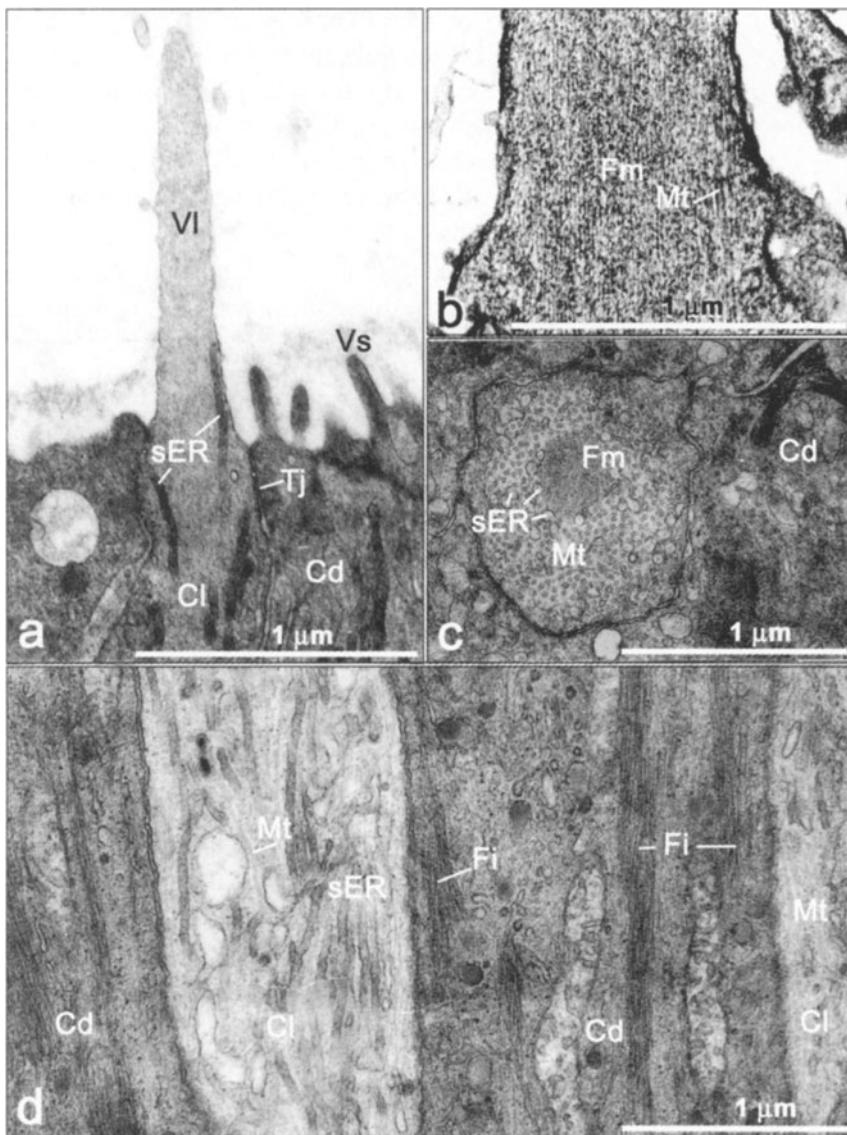
Fig. 5 (a-d): Transmission electron micrographs (as figs. 6 and 7, too) of the apical, subapical and supranuclear portions of fish tase buds.

(a): *Silurus* taste bud; longitudinal section of a large receptor villus (Vl) and some small receptor villi (Vs) belonging to light cells (Cl) and dark cells (Cd). sER - smooth endoplasmic reticulum, Tj - tight junction. (b): *Neoceratodus* taste bud. Basal part of a longitudinally cut large receptor villus that contains parallel and longitudinally running microfilaments (Fm) and, laterally, a microtubule (Mt). (c): *Neoceratodus* taste bud, cross section of its subapical region. A light cell centrally contains a bundle of microfilaments (Fm) surrounded by numerous microtubules (Mt) and some profiles of tubular smooth endoplasmic reticulum (sER). All these organelles are cross-sectioned. Cd -dark cell. d): *Astyanax* taste bud; longitudinal section of its supranuclear region with light cells (Cl) and dark cells (Cd) oriented in parallel. The light cells are rich in tubular smooth endoplasmic reticulum (sER) and microtubules (Mt). The dark cells show bundles of intermediate filaments (Fi). Scale bars: (a-d) 1 μm. c) by courtesy of Dr. Friederike Boudriot.

This and the fact that a part of the synaptic vesicles contain serotonin (Reutter 1971; Nada and Hirata 1977; Toyoshima et al. 1984) and the cell is positive to neuron specific enolase (NSE; (Toyoshima 1989) led to the interpretation that the basal cells might be related to Merkel cells (which belong to the paraneurons; see Fujita et al. 1988). Therefore, they were named "Merkel (cell)-like basal cells" (see Roper 1994; Zaccone et al. 1999; Zaccone et al. 2001). The function of basal cells is not yet clear. They might be a kind of interneurons in the bud; possibly they do have a paracrine function or may serve in chemoreception and/or mechanoreception (Reutter and Witt 1993). In any case they have nothing to do with the basally situated regenerative or proliferative cells: these are the basally located marginal cells (see below).

The **nerve fiber plexus** consists of unmyelinated dendritic axons ("nerve fibers") and the basal slender processes of the elongated cells of the sensory epithelium (Fig. 6). All these structures intermingle with each other intensely and make synaptic contacts. The elongated cells of the sensory epithelium must be regarded as secondary sensory cells because of the synapses at their bases. Also, the basal cells synapse to the nerve fiber plexus. Usually, synapses show only poor cytological details like membrane specializations and synaptic vesicles. Insofar, fish synaptology is not easy. Afferent synapses occur between the processes of light cells and the axons (Fig. 7a), between the dark cells and the axons (seldom) (Fig. 7b) and the basal cells and the axons (Fig.7c). Light and dark cells might be afferently connected to the basal cells (*Amiurus*: Reutter 1971, 1978). Efferent synapses are rare and occur between the axons and the light cells (*Amiurus*: Desgranges 1966; Royer and Kinnamon 1996; *Phoxinus*: Jakubowski and Whitear 1990; *Lepisosteus*: Reutter and Boudriot 2000; *Astyanax*: Boudriot and Reutter 2001).

This general description of a fish TB is not absolutely valid for all species or systematic groups of fish. For instance, hagfish and lampreys do not have TBs (see above; Baatrup 1983; Georgieva et al. 1979; Braun 1998). In selachians, TBs may occur, but their basal cells do not lie directly at the bud's base (*Scyliorhinus*: Reutter 1994; Whitear and Moate 1994b). TBs also may be lacking entirely (*Raja*: Whitear and Moate 1994a). The holosteans *Amia* and *Lepisosteus* show diverse types of receptor villi (Reutter and Boudriot 2000). In the teleosts *Astyanax* (Boudriot and Reutter 2001) and *Danio* (Hansen et al. 2002b) an additional (light?) cell type was detected. Further, there are structural differences between the basal cells of different species (see above). In short, fish TB ultrastructure, especially in view of cell types, seems to be taxon related and even species specific. It is likely that a real "fish TB type" does not exist (Reutter and Witt 1999; Reutter and Boudriot 2000).

Cell Turnover in Fish Taste Buds

Only one paper exists dealing with the cell turnover in TBs. Raderman-Little (1979) investigated the problem in *Ictalurus* by using H^3-thymidine autoradiography. Cells with nuclei H^3-thymidine labeled first were observed in the basolateral region of a TB where marginal cells are located. Later on, marked cells were found at the TB's base, and then in the middle part of the TB. Finally, marked nuclei also occurred in the upper third of TBs. It is hypoth-
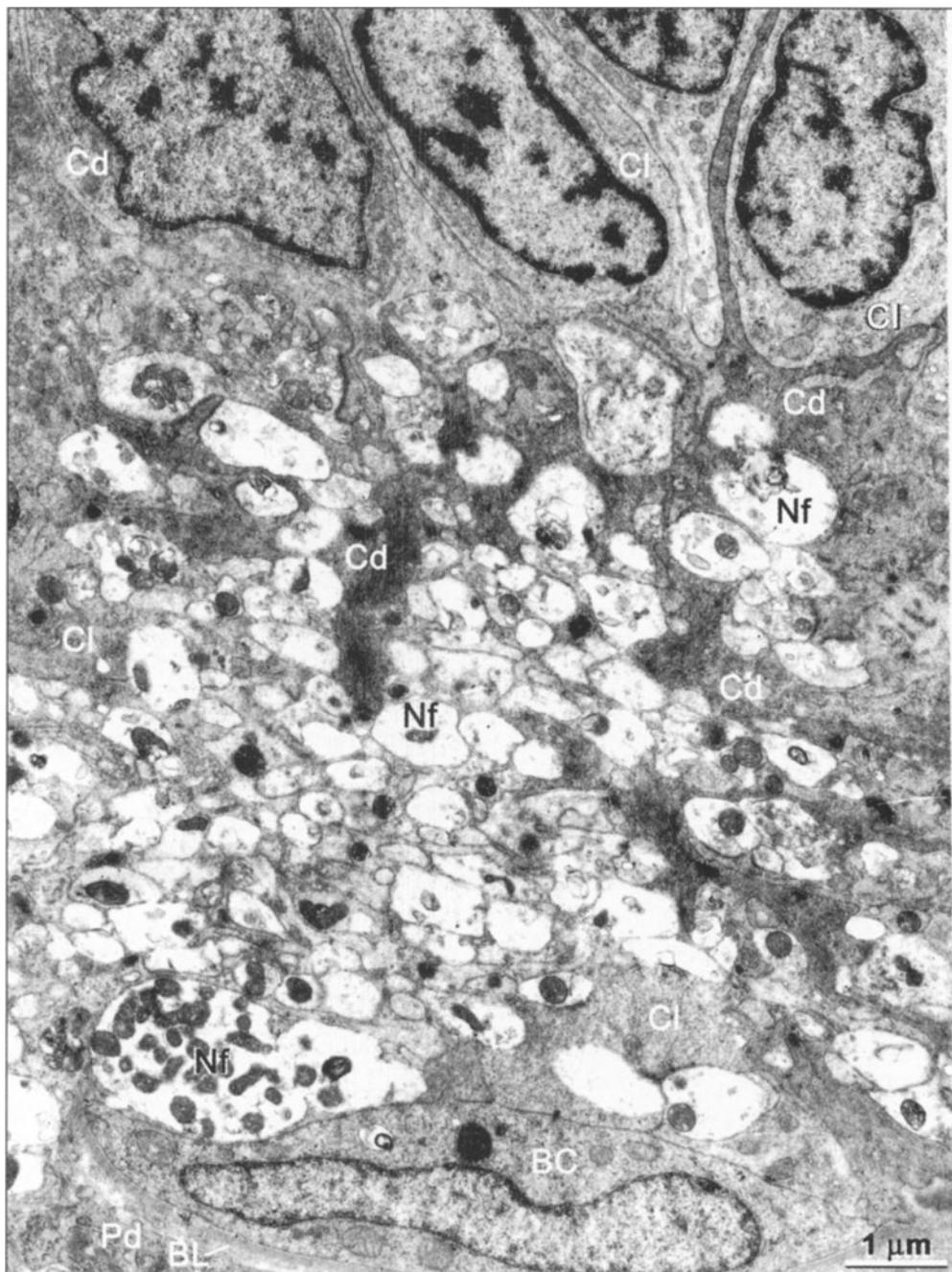
Fig. 6 *Plotosus* taste bud; longitudinal section through its basal part.

Above the light (Cl) and the dark cell's (Cd) bases. In the middle and lower part the basal processes of the light and dark cells intermingle with the axons (nerve fibers, Nf) of the nerve fiber plexus. Below, a basal cell (BC) sitting atop the basal lamina (BL). Pd - dermal papilla. Scale bar: 1 μm.
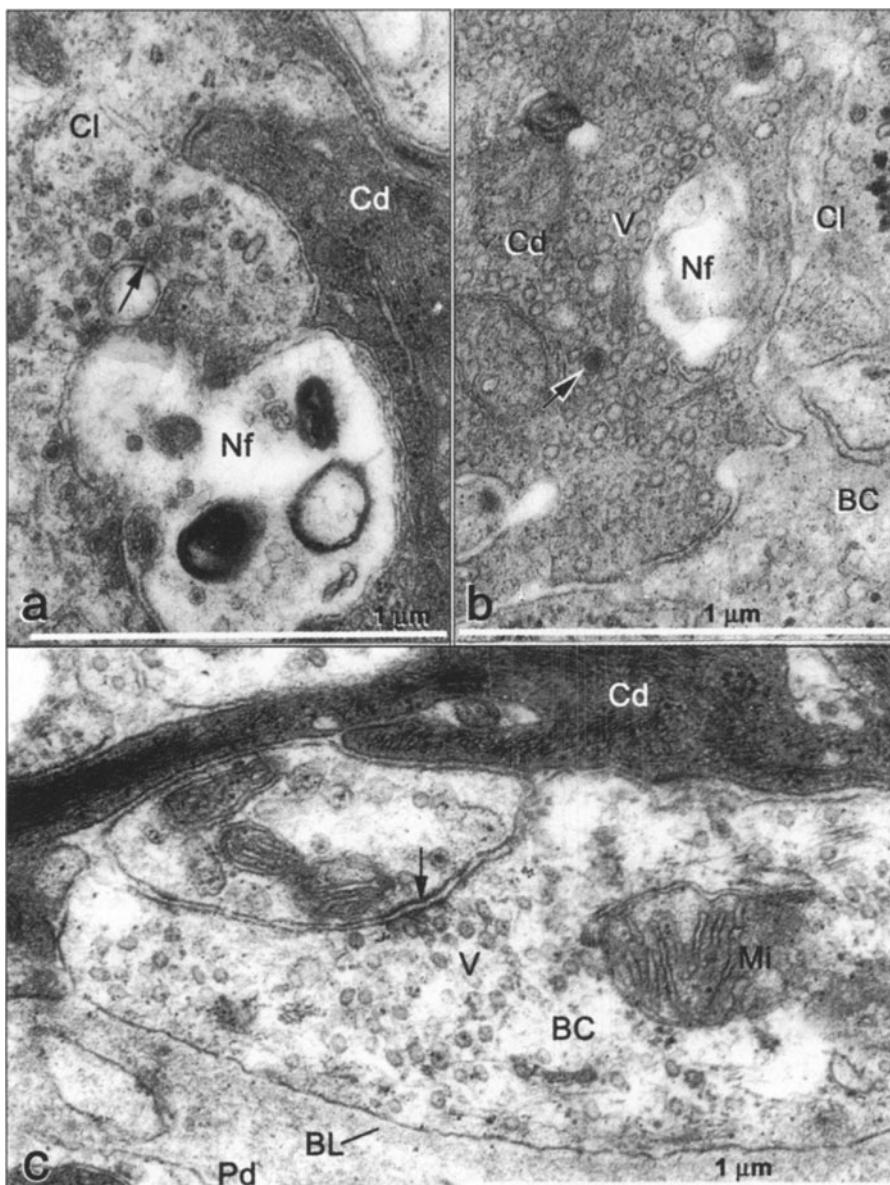
Fig. 7 (a-c): Longitudinal sections of synapting cells at the bases of fish taste buds.

(a) *Astyanax* taste bud. Small synapse between a light cell (Cl , presynaptic side) and a nerve fiber (Nf). Note the less pronounced membrane thickenings (arrow) and the small clear and the large dense cored vesicles (V) on the cell's side. Cd - dark cell. b): *Silurus* taste bud. A dark cell (Cd) containing numerous clear vesicles (V) and one large dense cored vesicle (arrow) is in synapse-like contact to an axon (Nf) and a basal cell (BC). Membrane specializations are poor. Cl - light cell. c). *Astyanax* taste bud. A basal cell (BC; presynaptic side) is rich in vesicles (V) and synapses to an axon of the bud's nerve fiber plexus. The arrow points to the well developed membrane thickenings. BL - basal lamina, Cd - dark cell, Mi - mitochondrium, Pd - dermal papilla. Scale bars: (a-c) 1 μm. a) and c) by courtesy of Dr. F. Boudriot.

esized that the marginal cells are the regenerative cells of a TB. They replace the oldest elongated TB cells in 30 days (18°C). Further, it is not yet clear whether from time to time the basal cells are also substituted by marginal cells. Possibly the basal cells are not of epithelial origin. It is discussed if they might be transformed glial cells (Schwann cells; Reutter 1978).

Innervation of Taste Buds and Gustatory Pathways in Fish

The axons of the TB's nerve fiber plexus are the distal dendritic endings of the first afferent neurons. These neurons form the peripheral part of the neuronal gustatory pathway. They exclusively run within one of the cranial nerves and, consequently, have their perikarya in the cranial ganglia of these nerves. (Nothing detailed is known about the morphological situation of the efferent taste nerve fibers).

As a rule, TBs situated in the anterior part of the buccopharyngeal cavity (lips, breathing valves, anterior tongue and anterior palate) and in the head's (especially on the barbels) skin are innervated by the facial nerve, the VII. cranial nerve. If there are TBs on the fins (including the tail fin) and on the flanks as in *Amiurus*, the TBs are innervated by the recurrent branch of the facial nerve (Herrick 1901; Finger et al. 1991). TBs of the posterior part of the buccopharyngeal cavity (posterior parts of the tongue and the palate, gill rakers and the entrance of the esophagus) are innervated by neurons that belong to the (small) glossopharyngeal nerve (IX.) and the (big) vagal nerve (X.). Both nerves together form the (glossopharyngeal-)vagal complex. All gustatory neurons project to the secondary gustatory neurons that lie in the oblongate medulla of the brain: The facial nerve projects to the facial nucleus or, when it is enlarged and protruding, to the so-called facial lobe as in the silurids and cyprinids (Finger 1976, 1978, 1997b; Kanwal and Finger 1992). The (glossopharyngeal-) vagal complex projects to the respective nuclei which together may build the protruding vagal lobe as especially in cyprinids (Morita and Finger 1985a, 1985b; Finger 1988, 1997b). Their secondary gustatory neurons are connected to the third gustatory neurons whose perikarya are located in the isthmic region, the posterior thalamic nucleus and the nucleus lobobulbaris. These third neurons may project to other diencephalic (and telencephalic?) centers.

Descending fibers from the secondary gustatory neurons of the vagal lobe terminate in the oblongate medulla (obex) and/or in the spinal cord. Further, secondary neurons of the vagal lobe may also be connected by interneurons to the motoric portion of this lobe. This fact seems to be quite important in view of food intake.

Gustatory sensory and motor vagal lobe neurons establish reflex circuits: if the food is edible and tastes well it is reflectorily swallowed, if not it is spit out or resurgated (Caprio et al. 1993; Finger 1997b). In the vagal lobe of the goldfish, *Carassius auratus,* this complex steering finds its morphologic substrate in a highly evolved multilayered system of neurons: the lateral layers are the sensory (gustatory) ones and are connected (by interneurons) to the vagal lobe's inner efferent (motor) layers (Finger 1997b; Morita and Finger 1985a; Morita and Finger 1985b).

Physiology of Gustation in Fish

It is well known that fish TBs, in contrast to most mammalian TBs, are not only sensitive to the popular sweet, sour, salty and bitter tastants (Glaser 1966) but also to a great variety of amino acids, nucleotides and bile acids (Marui and Caprio 1992). In fish, the threshold concentrations for detecting taste substances are far lower than in other vertebrates as amphibians or mammals (Marui and Caprio 1992). For instance, in the behavioral test the minnow, *Phoxinus phoxinus*, recognizes saccharose diluted down to 1.5×10^{-5} M, whereas the human saccharose threshold is 1×10^{-2} M. The bitter tasting quinine hydrochloride is detected at 4×10^{-8} M by fish and at 1.6×10^{-5} by humans. The low threshold concentrations for L- amino acids, especially L-alanine and L-arginine (1×10^{-5} to 5×10^{-8} M; electrophysiological test, Marui and Caprio (1992)) are typical for silurids and cyprinids which also feed on small animal cadavers that release the amino acids. The putative arginine receptor of *Ictalurus punctatus* TBs was demonstrated lectin- and immunohistochemically by Finger et al. (1996).

In fish, the threshold concentrations of amino acids vary in different fish and seem to be species specific. In channel catfish, *Ictalurus punctatus*, the most effective amino acids are L-alanine > L-arginine > L-serine > L-aminobutyric acid > L-glutamine (Caprio 1975). In the carp, *Cyprinus carpio*, the sequence is: L-proline > L-alanine > L-cysteine > L-glutamate (Marui et al. 1983). Even in different strains of a fish species the sensitivity to different amino acids can be different, as in rainbow trout, *Oncorhynchus mykiss* (Hara et al. 1999). What is more, the TBs situated in different parts of a fish (external/internal TBs, buccal/pharyngeal TBs) show species specific differences regarding their excitability by different tastants (Kanwal and Caprio 1983; Kasumyan 1997).

In nature, single chemicals are seldom an exclusive stimulus for taste receptors – mostly mixtures of different chemicals are to be detected. In the sea catfish, *Arius felis*, binary mixtures of amino acids lead to taste responses that correspond to the thresholds of pure amino acids. The mixtures do not cause the suppression of any of the tastants (Kohbara and Caprio 1996).

At the molecular level, recognition of tastants takes place especially at the cell membranes that cover the large and small receptor villi of the TB epithelium's elongated cells. These cell membranes contain the molecular receptors (receptor proteins) to which the different tastants bind. The binding of a tastant's molecule to its receptor initiates taste transduction. For the details involved, see Glendinning et al. (2000).

Taste System and the Fish's Environment

As said above, some fish that live in the dark possess more TBs in the oropharyngeal cavity and in the outer skin than fish of clear and well-lit waters do. So we expect less TBs in fish which are light active predators that hunt especially under sight control. But, as it is the case for the largemouth bass, *Micropterus salmoides*, a visual hunter, TBs are also numerous and positioned quite near to the teeth's bases, obviously ready for immediate testing of the prey (Linser et al. 1998). In general, fish living in the dark with huge amounts of TBs mostly

possess brains with enlarged gustatory brain centers as the facial and vagal lobes (see above). Interestingly, in some bottom-living deep-sea fish the relative volumes of their gustatory brain areas are above the corresponding values of non-demersal fish (Wagner 2002 and this volume). Such fish brains might be named "chemo-sensory brains", in contrast to "generalized brains" (Kotrschal and Palzenberger 1992), as they are typical for fish with "normal" gustatory abilities.

At the SEM level, Meyer-Rochow (1981) compared the structure and number of tongue TBs of mesopelagic and deep-sea fish and found TBs in large numbers in all species tested. Surprisingly, the tongues of deep-sea fish, living in the total dark, had relatively few TBs (*Sternoptyx diaphana* from 2000 m depth and *Diretmus spec.* from 700 m depth) with the exception of *Cataetyx memorabilis* (from 1300 m depth). All mesopelagic and benthic fish had less TBs than the trout, *Salmo (Oncorhynchus) gairdneri*. It is assumed that the number of TBs is species specific and correlated with the variety of food types a fish can access in its particular environment.

The latter point of view is corroborated by the growth characteristics of the reef fish *Upeneus tragula* (McCormick 1993). In this fish the sensory barbels and their TBs were examined from their appearance at early pelagic planktonic life until the reef-associated juvenile period. While the planktonic larvae had relatively small barbels and small TBs, the barbels grew (51%) and the mean size of TBs and TB cells increased (100%), too, during the 6-12 hours lasting period of settlement to the reef. Moreover, the mass of TB bearing epithelia was enlarged. Obviously, these changes are correlated with the newly metamorphosed fish's benthic life and its new situation of searching for food.

A good example for comparing the TBs of closely related fishes that inhabit quite different ecological niches is given by the Mexican genus *Astyanax* (Characidae). Since the Pleistocene the epigean river fish *A. mexicanus* gave rise to several populations, some of which now inhabit distinct caves of Mexico. One of these hypogean fish is *A. jordani*, formerly named "*Anoptichthys*", which lost vision. Accordingly, *Anoptichthys* and *Astyanax* offer the possibility for direct comparison of a cavernicole fish and its recent epigean ancestor. Such comparisons were extensively done for all the sensory systems including the gustatory system (Schemmel 1967, 1973, 1980). In *Anoptichthys*, the external TBs are more numerous and occur in larger skin areas than in *Astyanax mexicanus*. As seen in the light microscope, there are no structural differences between the TBs of both fish. Boudriot and Reutter (2001) compared the TBs at the TEM level. Overall, TB ultrastructure is rather similar in both fish, but the nerve fiber plexes of *Anoptichthys* type II and type III TBs contain significantly more axon profiles than those of *Astyanax*. This possibly means that the cave fish compensates for blindness by enlarging the site for synaptic transmission of gustatory signals.

Water Pollution and the Sense of Taste in Fish

There is no question that detergents and pollutants in the waters are affecting not only olfaction but also the taste system (and the solitary chemosensory cells, for that matter). In general, the facts regarding this problem are valid for both the olfactory organ (see above)

and the taste system. In the olfactory epithelium, the olfactory knobs and their substructures are directly exposed to the surrounding waters, and this is also the case for the receptor villi of the TB's receptor area (Bardach et al. 1965; Sutterlin 1974). For instance, decrease of the water's pH (from 8 to 6) significantly reduces the response to chemical feeding stimuli in the fathead minnow, *Pimephales promelas* (Lemly and Smith 1986). In acidic water possibly the thin superficial mucus layer atop the TB's receptor area (Bannister 1974; Reutter 1980) (and the olfactory epithelium, see above) is washed off and the microvillar membranes and their receptors resp. are then directly exposed to the environment. Also, traces of heavy metals affect the TBs. In the goldfish it was shown histochemically that lead ions (10^{-3} M) especially affect the mucus cells. Mercury-, copper- (10^{-4} M), and zinc ions (10^{-3} M) permeate TBs within 30 min of exposure and damage at least one (which one?) type of TB cells (Vijayamadhavan and Iwai 1975). The concentrations of metal ions used in these tests may be unrealistically high for polluted free waters. But we do not have any idea what will be caused by low heavy metal concentrations over a long lasting period of exposure, as it is the case in (industrially) polluted rivers, lakes and the sea.

Food Detection and Food Processing in Fish

To detect food fishes use several sensory systems, as olfaction, taste, vision and possibly also auditism, the lateral line system and electroreception (Valentincic and Caprio 1994, Valentincic this volume). For instance, the largemouth bass is gulping the prey under sight control whereas the ultimate decision of acceptance or rejection is done by the taste system, especially by the densely arranged TBs of the buccopharyngeal cavity (see above; Linser et al. (1998). Other fish find the food by olfaction, and food located near the fish by the external TBs, as on the lips and/or the barbels. Interestingly, the maxillary barbels of the channel catfish, *Ictalurus punctatus*, contain bimodal (tactile/taste) nerve fibers that point out the importance of mechanoreception and the double-function of the TBs discussed above Ogawa et al. (1997). Uptake of food and its further processing is different in different species and groups of fish. Osse et al. (1997) distinguished 4 modes of food ingestion: biting, ram feeding, side snapping and suction feeding. In contrast to the other modes, suction feeding and the further "handling" of the food is well understood thanks to the multitechnical investigations done especially in the carp, *Cyprinus carpio*, by Sibbing (1982); Sibbing and Uribe (1985); Osse et al. (1997). Carps as benthic omnivores, feed by suction with their protrusible and then enlarging tube-like orobuccal cavity. They possess toothless jaws, a dorsal palatal and a ventral postlingual organ in the anterior pharynx and, laterally, the gill rakers and their pads. Between these structures, only a slit-like gap remains. This is the place where the palatable food fraction of the material the fish took up by suction from the ground is separated from its organic and inorganic waste. This sorting requires a dense innervation pattern (mechanoreceptors), a dense and distinct arrangement of muscles and muscle fibers, and numerous TBs: as mentioned before, the palatal organ contains up to 820 TBs per mm^2! In the posterior pharynx (comprising the chewing cavity with the dorsal chewing pad and the ventral pharyngeal teeth) the food particles first are wrapped with mucus and then

masticated. Finally, the minced food is transported to the esophagus. These processes may be repeated for several times and finally lead to swallowing of edible foodstuff or to spitting out of waste or non-tasting substances. This all requires a well elaborated steering system that works with a viscerosensory and gustatory input and a detailed visceromotor output. It is equipped with numerous feedback loops between afferent and efferent pathways (Finger 1997b). Insofar it is not astonishing that the vagal lobe of cyprinids is highly developed allowing multiple and fast reflex circuits (see above).

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Taste and Olfactory Stimuli and Behavior in Fishes

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ABSTRACT

When searching for food different fish species use the same sensory mechanisms differentially. At one extreme there are omnivorous fishes such as catfish and carp that, in addition to vision, use the taste system to excite and release reflex responses and the olfactory system to excite and discriminate chemical stimuli. On the other extreme are predatory fishes that detect prey visually and, if not conditioned differentially at fry and fingerling stages, do not use chemosensory information for food finding at all. Depending on the mechanisms that they use to detect and collect food, the fishes that have been studied to date occupy the following ecological niches. (1) Bullhead catfish (*Ameiurus melas*) type omnivorous niche: bullhead catfishes use chemical and tactile senses to release appetitive and consummatory phases of feeding behavior. They also detect prey by the passive electric sense. (2) Channel catfish (*Ictalurus punctatus*) type omnivorous niche: channel catfish use vision for predation and, in addition, chemical and tactile senses. (3) Carp (*Cyprinus carpio*) and goldfish (*Carrasius auratus*) type omnivorous niche: carp use visual and chemical senses for food collection, they have taste-controlled reflex snapping/biting mechanisms and, in addition, they use oral food sorting to separate edible from inedible objects. (4) Rainbow trout (*Oncorhyncus mykiss*) type visual hunters niche: farm-raised rainbow trout use vision and/or olfaction to get excited and search for food. (5) Exclusively visual hunters niche: in nature, European huchen (*Hucho hucho*) and walleye (*Stizostedion vitreum*) consume exclusively living prey such as fish and crustaceans that they locate by vision.

Physiologically functional olfactory (Cooper and Hasler, 1976; Shoji et al., 1994) and taste organs (Marui et al., 1983) do not necessarily indicate that a predatory fish uses either olfaction or taste to find food. In nature, visual hunters such as huchen and walleye do not get excited by taste and olfactory stimuli, they neither bite/snap after taste stimulation nor do they use olfaction to discriminate chemical stimuli. In most predatory fishes the taste system is used solely during oral food evaluation. At fry and early fingerling stages, huchen and walleye can learn to eat non-living foods such as minced liver and industrial starter feed. Juvenile walleye were conditioned in a first step to eat non-living food, to respond to olfactory stimuli in a second step and, in a third step, to discriminate amino acids. Thus, early learning influences the functional expression of the nerve networks that enable the use of olfactory information in the control of feeding in predatory fish.

Key words: Feeding behavior, Olfactory stimuli, Taste stimuli, Chemo sense, Olfactory learning

COMPLEX FEEDING BEHAVIOR

Feeding behavior is composed of orienting responses, appetitive search swimming, reflex turning and consummatory behaviors such as reflex snapping/biting, oral manipulation, mastication and swallowing. In most cases omnivorous fish detect their food by either visual or chemical senses. Visual stimuli enable fish to swim directly at food items without the need of chemical stimulation, whereas olfactory or taste stimuli excite fish to swim around in search for food (Valentinčič and Caprio 1994). In nature, many carnivorous fish are exclusive visual hunters that do not use chemical stimuli to locate and bite at food.

Which chemical stimuli enable fish to locate non-living food? The most likely candidates are low-molecular water-soluble compounds such as amino acids. Amino acids are the building blocks of all living organisms. They are present in proteins and free amino acids are dissolved in the cytoplasm. Amino acids leak from living organisms and from carrion. The concentration of free dissolved amino acids in water actually depends on the balance between loss and uptake of amino acids from and into living organisms (Fergusson 1980). The concentrations of free dissolved amino acids in seawater, such as L-alanine and glycine, are greater than 100 nanomolar, whereas concentrations of unstable amino acids such as L-cysteine in natural waters may be as low as one nanomolar. Those animals that detect increases in amino acid concentrations above natural background concentrations potentially detect the presence of food. Besides the sensitivity for amino acids researchers reported sensitivities of fish chemoreceptors for aliphatic acids, nucleotides and bile salts (Marui and Caprio 1992). The presence of these substances can also indicate food, however their precise action on fish behavior is not known.

Two main processes distribute chemical stimuli in water: diffusion and currents. At distances below 0.1 millimeter diffusion is an extremely rapid process. In contrast, at distances larger than centimeters diffusion is an extremely slow process (Table 1; Jacobs 1934). At the scale of bacteria and small protozoa diffusion is the rate-limiting process for stimulus distribution, whereas at the scale of fishes water currents are the rate-limiting mechanism. In the world of microorganisms regularly shaped diffusion gradients surround the stimulus source, whereas in the world of fishes chemical stimuli are irregularly distributed in space. On the fish scale water currents carry around bodies of irregular shapes called eddies (turbulent odor plumes) that might contain high concentration of chemical stimuli; each eddy is larger than several centimeters. The thin peripheral layer of the eddy empties very quickly into the environment whereas its interior is centimeters away from the surrounding water; the high concentration of chemical stimuli within the eddy is preserved for several minutes.

Fish swimming through eddies with a speed of > 0.2 m/sec encounter high concentrations of chemical stimuli intermittently (Fig. 1). When a fish swims through an eddy, taste and olfactory receptors are bathed with high concentrations of chemical stimuli for a fraction of a second only. The nasal ciliary pumping mechanisms create rapid water currents between the olfactory lamellae, but these mechanisms do not limit the duration of the olfactory receptor cell exposure

to chemical stimuli. The olfactory receptors are bathed in stimulating solutions at the rate of encounters with the stimulus eddies (Fig. 1). No adaptation of chemoreceptors occurs, the fish stays alert of chemical stimulation for as long as the high stimulus concentration eddies exist.

Table 1 (from Jacobs, 1935): Diffusion at a short distances is an extremely rapid process, less that 50 milliseconds are necessary to reach a steady state at a distance of 10 micrometers from the boundary between the two layers, at large distances such as one centimeter diffusion is an extremely slow process, twelve hours are necessary to reach steady state at this distance.

Distance	Time
1 cm	12.72 hrs
1 mm	7.6 min
100 μm	4.56 sec
10 μm	0.046 sec
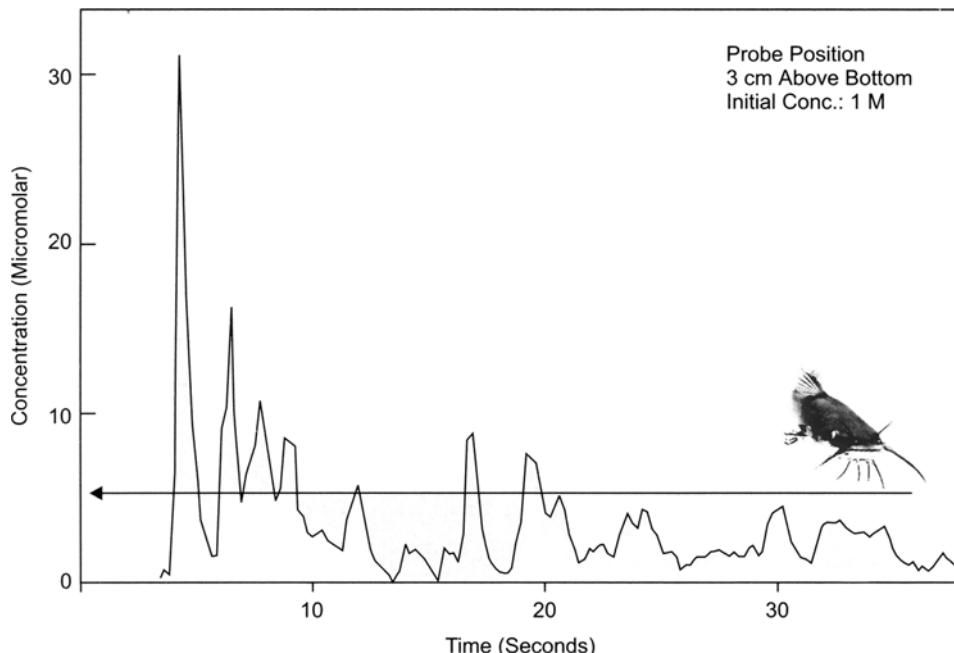
Fig. 1 Aquarium aeration provides mixing that brings eddies (turbulent odor plumes) containing high concentrations of the stimulus (the odor model substance was dopamine) over the redox probe (Moore and Atema 1988; Moore et al. 1989). Dopamine was injected at 1 molar concentration; its dilution within the first eddy that passed the probe was 30000 times. Arrow indicates a potential swimming direction of a bullhead catfish that crosses the high stimulus concentration eddies. Each eddy passed the redox probe in few seconds, at a speed of 0.2 m/s a fish swims through a 5 cm eddy in approximately 250 milliseconds.

FEEDING REFLEXES ARE RELEASED BY TASTE STIMULI

In catfish chemical stimuli such as L-proline, L-alanine and L-cysteine release two simple reflex responses, turning and snapping/biting behavior. Snapping and biting behaviors of catfish are essentially the same reflex behavior; chemical stimuli alone release the snapping behavior which is a large opening and closing of the mouth, whereas the biting behavior occurs due to either chemical or tactile stimuli alone or due to both stimuli presented simultaneously. The snapping/biting responses were studied in anosmic channel (*Ictalurus punctatus*; Valentinčič and Caprio 1994) and black bullhead (*Ameiurus melas*, the European populations of this imported species were long considered to be *A. nebulosus*) (Valentinčič and Pirc, unpublished; Fig. 2A and B) catfishes. The threshold for the snapping/biting responses to L-alanine is slightly above micromolar (Fig. 2C) and it is above 100 micromolar for L-proline. In the case of L-proline the behavioral threshold value corresponds closely to its electrophysiological threshold concentration (Caprio 1978; Valentinčič and Caprio 1994). When an anosmic catfish encounters eddies that contain supra-threshold amino acids for the facial taste, it turns to the side of the more stimulated barbel. The tropotactic turns are not erroneous at millimeter distances upstream and at sides from the stimulus source and few centimeters downstream from it. Due to the irregular distribution of eddies that contain high concentrations of amino acids the first tropotactic (Fraenkel and Gunn 1940) turn usually does not bring the catfish close to the stimulus source. Many turns are needed and the entire swimming path of the catfish resembles klinokinesis rather than tropotaxis. During klinokinesis an organism changes the direction of swimming when the concentration of an attracting stimulus starts to decrease. Sooner or later the increased swimming and turning activities brings the catfish to the stimulus source.

There is a difference between channel and black bullhead catfishes in responses to L-arginine. L-arginine releases snapping/biting reflex in channel catfish, whereas bullhead catfish responds little to this amino acid (Fig. 2A and C). High concentrations (>10 millimolar; Valentinčič and Caprio 1994) of L-arginine trigger mastication in the channel catfish. The mastication consists of movements of the hyoid region of the catfish. L-arginine does not release mastication in the bullhead catfish. It appears that bullhead catfishes lack both the high and the low affinity arginine receptors that were described in the channel catfish (Kalinoski et al. 1989).

ORAL FOOD SORTING BEHAVIOR OF GOLDFISH AND CARP

Goldfish and carp also respond to L-alanine and L-proline stimuli with snapping/biting reflexes (Lamb and Finger 1995). Goldfish and carp snap/bite even before they start to swim in search for food. Several snaps/bites occur during an encounter with the first stimulus eddy; further eddies with amino acids release few additional snaps. After the snapping/biting behavior brings food into the mouth, oral food sorting, swallowing and pharyngeal mastication may follow.

Initially goldfish and carp suck water and sediment into the mouth and in the next step oral food sorting behavior separates edible from inedible particles. The snapping/biting and sucking activities of goldfish and carp is controlled in the facial lobe whereas the food sorting activity is
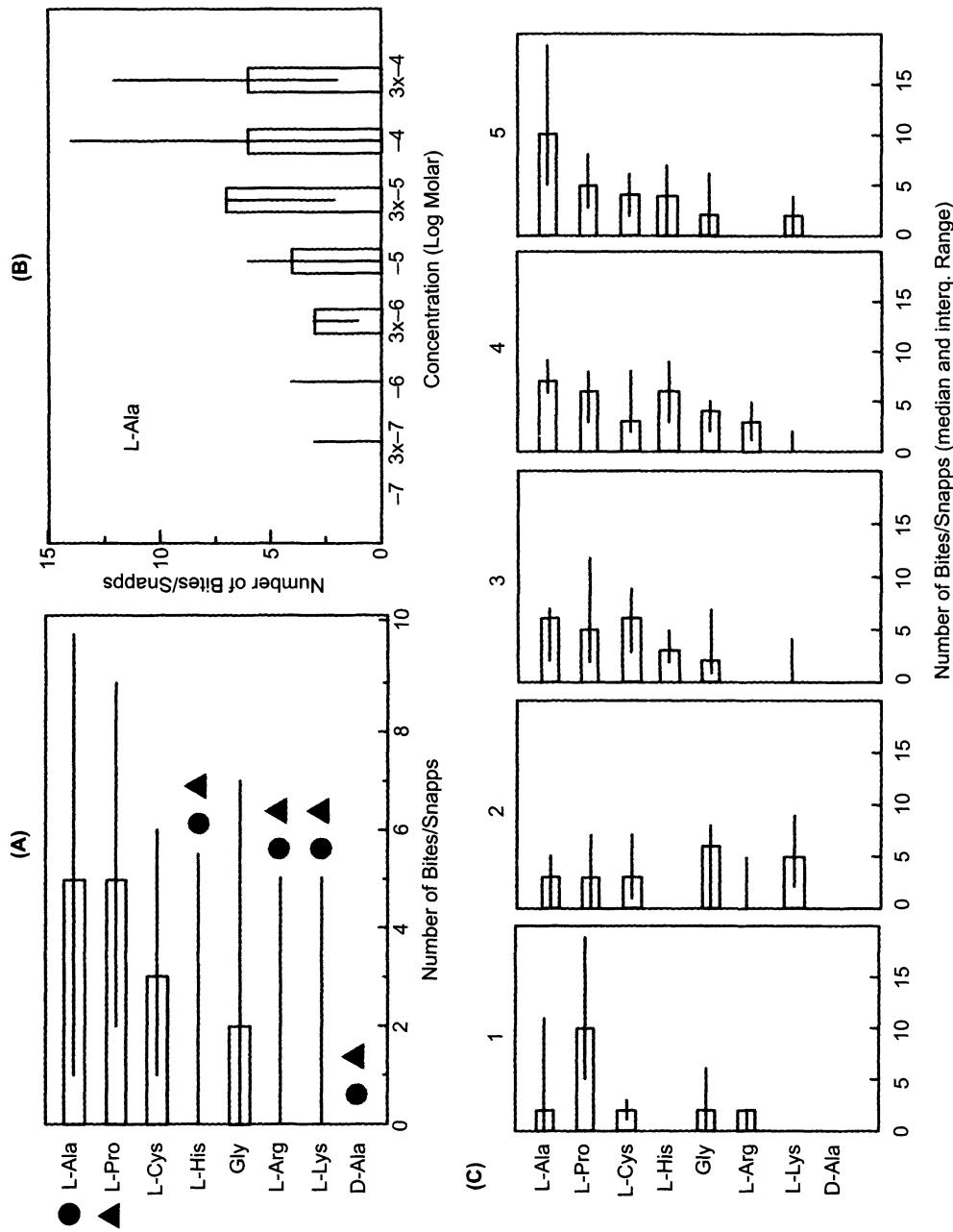
Fig. 2 Snapping/biting in anosmic black bullhead catfish. (A) The most effective snapping/biting stimuli for the black bullhead catfish. (B) Snapping/biting effectiveness of L-alanine indicates its behavioral taste threshold at above 1 micromole. (C) Five series of tests conducted at intervals of 14 days revealed high repeatability of amino acid snapping/biting behavior. Dots and triangles indicate significantly lower numbers (P>0.05) of snapping/biting responses compared to L-Ala and L-Pro responses respectively.

controlled in the vagal lobe. Separate sensory and motor nuclei control oropharyngeal food sorting, pharyngeal mastication and visceral responses (Goehler and Finger 1992; Finger and Dunwiddie 1992). The specialized orobranchial subsystem that controls sorting of sediment from food is located in the laminated vagal lobe of goldfish that contains primary sensory terminals, interneurons and motor neurons (Finger 1988). The size of the anatomical structures that support oral food sorting is very large suggesting complex behavioral tasks. The oral food sorting behavior is composed of rinsing and backwashing (Sibbing 1982; Sibbing et al. 1986; Sibbing 1991; Lamb and Finger 1995). During rinsing and backwashing the water passes over the particles held between the palatal and post-lingual organs in forward and in backward directions. The complex patterns of contraction of bucal musculature resemble, such as swallowing (Doty and Bosma 1956), fixed action patterns. The stimuli that release rinsing and backwashing are supposedly chemical and tactile, however, it is not know if both kinds of stimuli release food sorting behavior only in conjunction or also when presented separately (Lamb and Finger 1995). Pharyngeal mastication and abdominal responses are controlled in ganglia at the caudal region of the vagal lobe.

TASTE STIMULI CONTROL FEEDING EXCITATION IN CATFISH

Taste stimuli alone excite anosmic catfish to start food searching behavior. Catfish have abundant connections of the facial lobe through the supramedullary gustatory pathways to the nucleus gustus secundarius and diencephalic regions of the brain (Finger and Kanwal 1992; Finger 1987; Kanwal and Finger 1991). Tests in anosmic channel (Valentinčič and Caprio 1994) and bullhead (Valentinčič and Pirc unpublished) catfishes have shown that anosmic catfish (Fig. 3) snap/bite at or few seconds after the start of the food searching activity. Taste stimulation alone creates a central excitatory state for feeding originating from the hypothalamic region of the diencephalon that supposedly has a function similar to the mammalian hypothalamus. The above statement is experimentally supported by data on the maximal swimming and turning activities that are nearly the same after L-alanine and L-arginine stimulation in anosmic channel catfish and after L-alanine stimulation in intact channel catfish conditioned to L-alanine (Valentinčič and Caprio 1994).

INHIBITION OF FEEDING BEHAVIOR BY ESCAPE BEHAVIOR AND FEAR

The amount of food searching activity depends on reward expectancy and on inhibition of feeding behavior from escape behavior. In catfish key stimuli for escape behavior release fear, escape dashes and freezing responses. Two related catfish species, channel and bullhead catfish, detect the key stimuli for escape behavior with two entirely different sensory systems. Channel catfish have large eyes and use vision to detect danger, whereas bullhead catfish have very small eyes and use low frequency vibrations to detect danger. In channel catfish objects of any shape that prevent the unobstructed view of the sky and views of white substrate completely inhibit feeding behavior (Valentinčič and Caprio 1994a). In bullhead catfish, in contrast, low frequency vibrations completely inhibit feeding behavior (Valentinčič et al., 2000a). There is

Fig. 3 To study taste control of snapping/biting and feeding behaviors olfactory organs of juvenile (less than two years old) channel catfish were extirpated. Within the period of one month connective tissue and skin covered the surgical lesion and the catfish remained permanently anosmic.

no behavioral sensitivity to low frequency vibrations in the channel catfish and no behavioral sensitivity to visual key stimuli for escape behavior in the bullhead catfish. What are the differences in the natural habitats of the two catfish species that account for these differences in escape behaviors? Both catfish species inhabit stagnant waters in North America, however, channel catfish also inhabit rivers such as the Mississippi. Probably large eyes and acute vision are an advantage for the successful survival of channel catfish in flowing water, whereas the detection of biologically relevant low frequency vibrations is probably impaired in flowing water that is a potential source of low frequency vibrations itself. Predators of catfish are different species of birds including herons that, when fishing, stand motionless in shallow water and watch for moving prey. This particular heron fishing method practically eliminates fishes that try to move under the watchful eyes of the bird. When a heron lands at its fishing spot, the channel catfish sees the bird above the water, dashes away and freezes on the bottom. The catfish remains motionless for many hours or even for days. This is a very successful method for survival of the catfish that cannot be detected by the motionless bird. Successful survivals have shaped the two different escape behavior mechanisms in the channel and black bullhead catfishes.

The duration of a food searching activity depends on the level of inhibition by fear and on the level of food reward expectancy. More than 30 conditioning sessions are necessary for an average catfish to regularly respond to the conditioned stimulus. In most cases catfish respond with more than twice the swimming activity to the conditioned stimulus versus the activity released by non-conditioned stimuli. To measure the swimming activity we either video-tracked (Vidmex V, Columbus Instruments Ohio) the distance traveled by the centroid of the fish (Fig. 4) or counted the number of turns of fish >90 degrees during swimming. Both methods yield highly correlated results, R for the channel catfish was 0.9-0.95 and R for the bullhead catfish was 0.75-0.85. The differences in correlation coefficients originated in different swimming patterns of the catfish species studied: the highly visual channel catfish mostly swam two dimensionally along the front glass of the aquarium whereas bullhead catfish swam in all the three dimensions around the aquarium.

Fig. 4   During 90 seconds after the L-alanine conditioned stimulus presentation highly active bullhead catfish search for food in a complicated swimming path that includes numerous turns above 90 degrees. The search swimming path is revealed by video-tracking.

LEARNING TO DISCRIMINATE OLFACTORY STIMULI – THE MOST IMPORTANT FUNCTION OF THE OLFACTORY SYSTEM

In catfish (Valentinčič et al. 1994; Valentinčič et al. 2000), carp and goldfish (Zippel et al. 1993; Hoyk et al. 1993; von Rekowski and Zippel 1993) the olfactory system enables the discrimination of amino acids. If an amino acid stimulus is associated with food reward, no inherent conflict is provoked by the experimental paradigm. Such experiments yield consistent results. On the contrary, when the experimenter associates snapping/biting stimuli with escape behavior, he creates inherent conflict between feeding and escape behaviors. The conditioned fish are confused and the behavior flip-flops between feeding and escape states. For this reason some results of the heart rate conditioning experiments that used amino acids to condition fear were contradictory (Little 1981; Holland and Teeter 1981). In our experiments catfish received food reward 90-120 seconds after the conditioning solution was injected into the test aquarium. The

fish encountered eddies containing high concentration of amino acids in less than 30 seconds after stimulus delivery and the high concentration of amino acids within the eddies persisted for nearly two minutes. The food search time was up to two minutes, and after stimulation with the conditioning stimulus catfish and carp swam significantly faster and longer than after stimulation with the non-conditioned stimuli.

Channel and bullhead catfish discriminated nearly every amino acid from every other amino acid. The only pair of compounds that channel catfish did not discriminate was L-proline and its analogue pipecolic acid (Valentinčič et al., 1994). Bullhead catfish did not discriminate the pair L-valine and L-isoleucine. These two amino acids supposedly modulate the activity of the same olfactory receptor neurons (Valentinčič et al. 2000). Some bullhead and some channel catfish also had difficulties to discriminate glycine and L-serine from L-alanine, which supports the notion of short-chain neutral amino acid chemoreceptors (Caprio and Byrd, 1984).

In nature, discrimination of mixtures occurs more frequently than discrimination of single compounds. We studied the behavioral discrimination of binary (mixture of two components), ternary (mixture of 3 components) and multi mixtures of 5-7 and 10-13 amino acids in bullhead and channel catfish. Catfish conditioned to amino acid mixtures composed of 2-3 components initially responded to the conditioned mixture and to its electrophysiologically most stimulating component (amplitude of the electroolfactogram, EOG) with the same intensity in food-search swimming (Fig 5). This indicates that both, the conditioned mixture and its most stimulating amino acid are initially perceived as the same odors (Valentinčič et al. 2000; Fig. 5). During discrimination training (more than five successive comparisons of the conditioned mixture and its most stimulating component) the catfish began to detect the fine difference between the mixture and its most stimulating component alone. The fine difference that originates from the small additional stimulation of the minor component in the mixture allowed the catfish to perceive the conditioned mixtures as different from its most stimulating component. Bullhead catfish conditioned to the multimixture of seven equally effective amino acids were able to discriminate the conditioned mixture from mixtures that contained four, five and six of the same amino acids (Kralj and Valentinčič unpublished). On the other hand, bullhead catfish were not able to discriminate the conditioned multimixture of thirteen equally effective amino acids from the multimixture of twelve amino acids, however, they detected multimixtures of eleven and ten amino acids as different from the conditioned multimixture of thirteen amino acids (Zgonik and Valentinčič unpublished).

REGENERATED OLFACTORY ORGANS ENABLE EFFECTIVE OLFACTORY DISCRIMINATION

Surgical extirpation of olfactory organs of adult catfish does not trigger an immediate growth of the connective tissue. The lesion remains open for nearly two months, and a functional regeneration of the olfactory organ was first confirmed after 80 days. The newly formed olfactory rosette stays exposed to the environment, and its size is considerably smaller than that of the intact rosette. In some cases only few olfactory lamellae regenerate. It is important to note that even a single lamella enables olfactory discrimination of amino acids that is as effective as

Response to Ternary Mixture Components
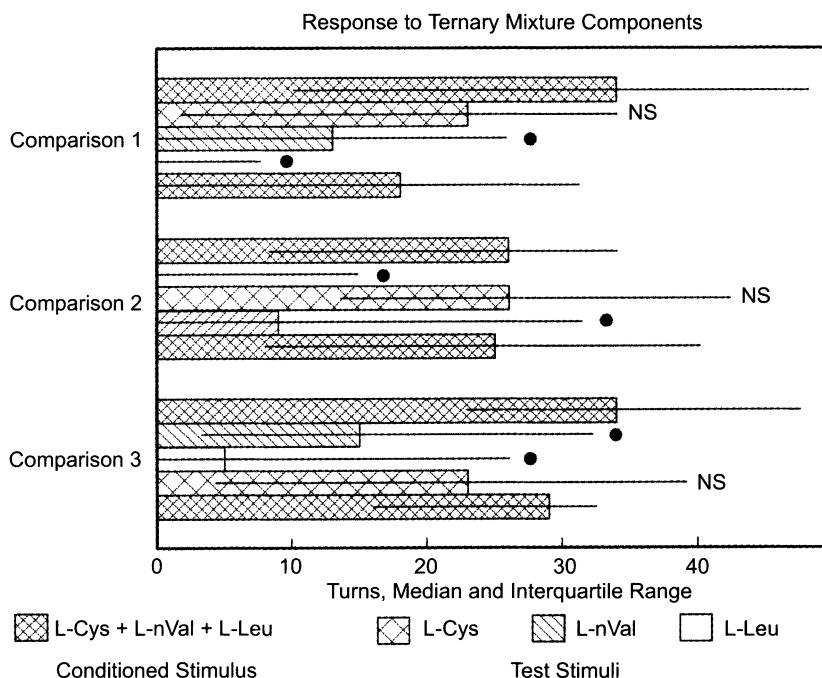
Fig. 5   During the first three comparisons bullhead catfish conditioned to the ternary mixture of L-Cys+L-nVal+L-
Leu with L-Cys as the most stimulatory component did not discriminate L-Cys from the conditioned
mixture and discriminated the other two components from the mixture. The conditioned ternary mixture
was tested twice, before and after the test stimuli.

the olfactory discrimination of amino acids in intact bullhead catfish (Stenovec and Valentinčič
2001). This is an indication that axons from the newly formed olfactory receptor cells
reconnected the olfactory bulb glomeruli in a fully functional chemotopic manner. Functional
regeneration of olfactory organs was reported also for goldfish (Zippel et al. 1993a; Hoyk et al.
1993; Rekowski and Zippel 1993; Zippel, et al. 1993b).

CHEMICAL SENSES AND FEEDING BEHAVIOR OF FARM-RAISED RAINBOW TROUT

Visual stimuli from prey, and conditioned visual stimuli such as hand of the feeder, release very
intense feeding behavior in rainbow trout. Contrary to other salmonids, adult farm-raised
rainbow trout respond with food searching behavior also to olfactory stimuli (Valentinčič and
Caprio 1997). In the absence of the chemical stimulation visual stimuli alone release the entire
sequence of feeding behavior: swimming and turning, snapping/biting behavior and ingestion.
No chemical stimuli are needed for snapping/biting behavior to occur as a part of the complex
pattern of feeding behavior. To create anosmic rainbow trout the olfactory organs of immature
rainbow trout (18-23 cm) were surgically removed under MS-222 anesthesia. Within one
month the surgical lesion was re-grown by connective tissue and skin, such as in bullhead and

channel catfishes, and the rainbow trout became permanently anosmic. In spite of the fact that the rainbow trout taste system is morphologically well developed (Meyer-Rochow 1981) and extremely sensitive to L-proline (Marui et al. 1983), the anosmic rainbow trout responded to this stimulus neither with the complex feeding behavior nor with the snapping/biting reflex. In anosmic and in intact adult rainbow trout the reflex responses to chemical stimuli are suppressed. Alevins, juveniles that obtain nutrients from their yolk sacks, do not respond to chemical stimuli with feeding behavior, there is no food searching activity and the little fish do not feed. However, the taste controlled snapping/biting reflex occurs regularly (Valentinčič et al., 1999). The complete snapping/biting reflex is observed in alevins before brain structures that control feeding excitatory state start to work for the first time (Fig. 6A). After the alevins start to respond to visual feeding stimuli, food-searching behavior begins and snapping/biting reflexes to L-proline and L-alanine stimulation are inhibited permanently. The snapping/biting behavior that is observed during feeding of anosmic rainbow trout is an integral part of the complex pattern of feeding behavior that is controlled directly by the brain (Fig. 6 B).
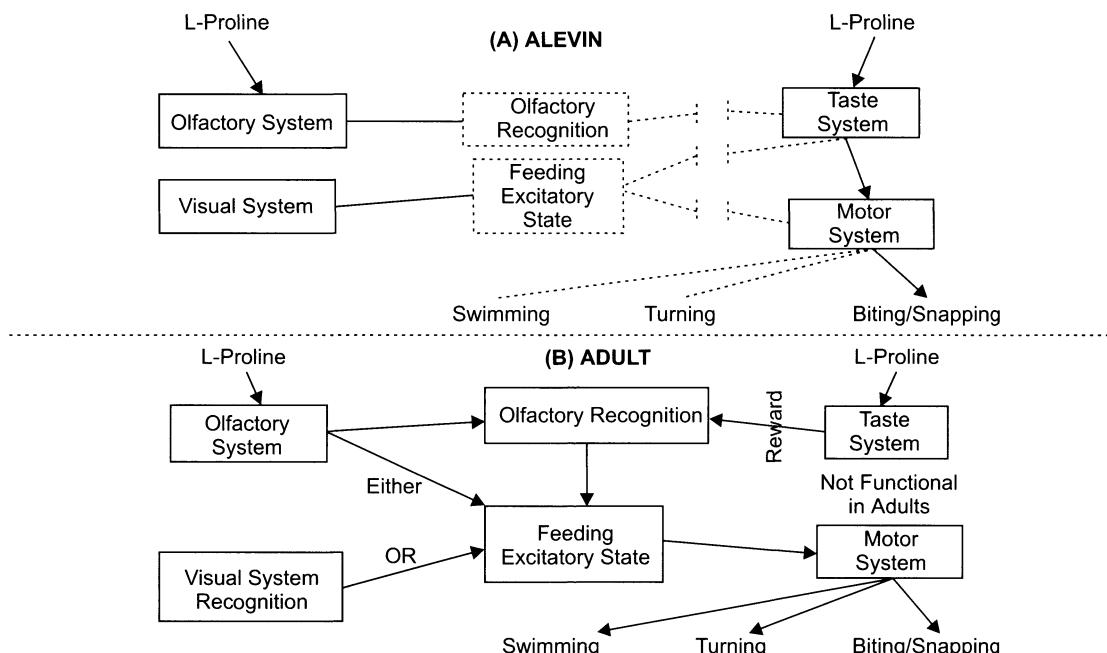
Fig. 6 Rainbow trout alevins (A) are not excited by feeding stimuli, however they snap and bite after L-proline and L-alanine stimulation. Intact adult rainbow trout (B) swim and search for food after olfactory stimulation; however anosmic animals do not respond to amino acid stimuli at all (adapted from Valentinčič et al. 1999). Solid lines and arrows indicate functional nerve networks, dotted lines indicate mechanisms and behaviors that do not occur in alevins. NOT FUNCTIONAL IN ADULTS indicates existing reflex connections; however, these connections do not enable reflex responses to chemical stimuli.

CHEMICAL SENSES IN EXCLUSIVELY VISUAL HUNTERS

In nature, the majority of European huchen (*Hucho hucho*; Salmonidae) and walleye (*Stizostedion vitreum*; Centrachidae) are exclusive visual hunters that do not pay attention to chemical stimuli. These fish capture swimming prey like little fish and crustaceans and swallow it whole. Such food items are bags of high-value proteins that do not leak or leak little dissolved amino acids. In experimental conditions farm-raised fingerlings of walleye (Fig. 7A) and fry and fingerlings of European huchen did not respond to chemical stimuli with feeding behavior. Most fish from nature that are visual hunters hardly ever bite/snap after stimulation with chemical stimuli alone.
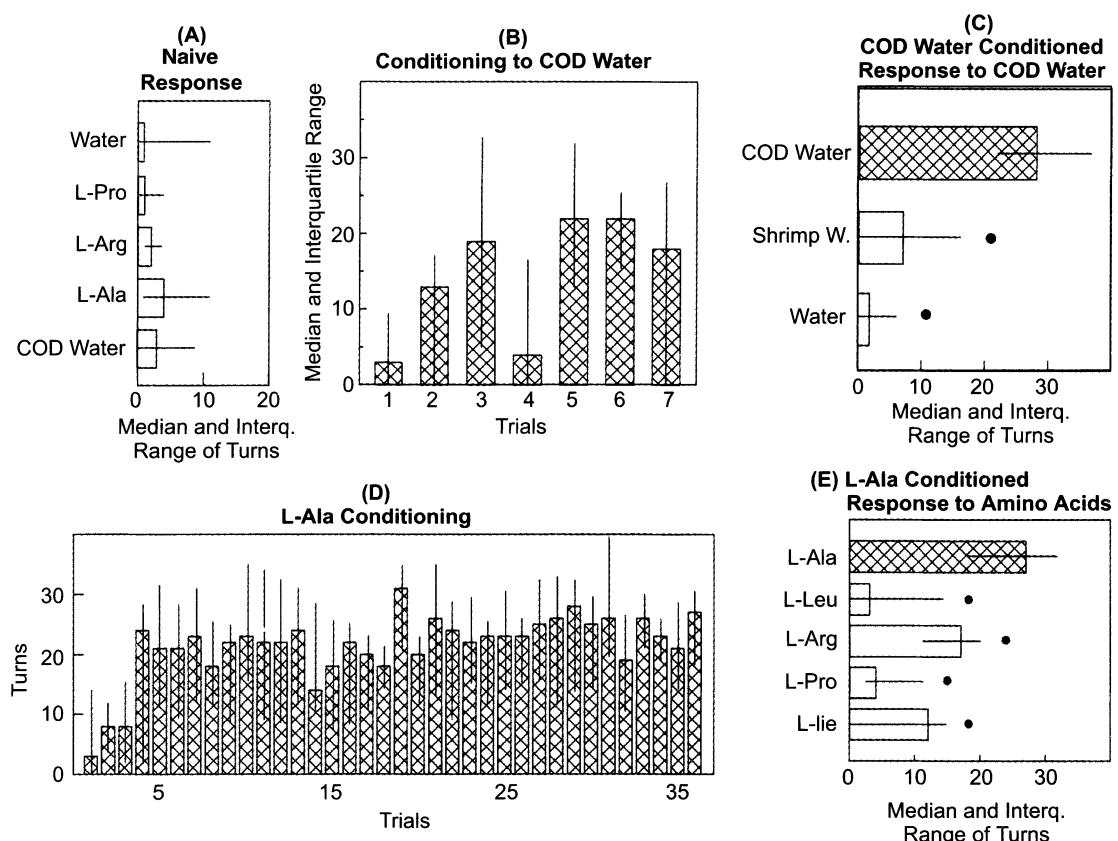
Fig. 7 Walleye were trained to eat cod muscle and industrial food pellets. These fish did not respond to cod water alone (A). The fish were than conditioned to receive food rewards after 90 seconds of cod water presentation (B). Walleye conditioned to cod water responded significantly more to the cod than to the shrimp water (C). Walleye conditioned to respond to cod water were later conditioned to respond to L-alanine (D). The walleye conditioned to L-alanine responded significantly more to L-alanine that to the other amino acids stimuli tested (E). White bars indicate median number of turns during swimming after stimulation with the non-conditioned stimuli, cross-hatched bars indicate turning responses to the conditioned stimuli. The error bars are for interquartile range of the number of turns (N=10).

CONDITIONING ENABLES EUROPEAN HUCHEN TO EAT NON LIVING FOODS

We trained 28 days old fry of European huchen (*Salmo hucho*) to eat non-living food. Initially the huchen fry received nauplii of brine shrimp *Artemia salina*. In later trials huchen were offered minced spleen in the morning and living enchitreid worms (*Enchitraeus albidus*) cut into halves at the end of the day. During the second week we used starter feed 000 in the morning, minced spleen in the afternoon and cut enchitreid worms in the evening. During the third week the quantity of the enchitreid worms was reduced to feed only those fry that did not accept the non-living food. Less than 20% of the huchen were lost by the end of the three-month food transfer period, all the remaining huchen ate industrial food pellets. In the fish farm Povodje near Ljubljana the experimental huchen were raised on industrial food pellets to maturity.

CONDITIONING ENABLES WALLEYE TO DISCRIMINATE L-ALA FROM OTHER AMINO ACIDS

In spite of the fact that walleye detect the amino acids L-Ala, L-Arg and L-Pro with their taste and olfactory systems (Caprio et al. unpublished), ten to fifteen centimeter fingerlings of walleye did not respond to cod water and single amino acids (Fig. 7 A). These fish also did not feed on non-living food such as cod muscle, carrion and industrial food pellets. To change their diet from living fish and crustaceans, the walleye were offered pieces of cod muscle and liver daily. To maintain their health they were fed with living minnows (*Phoxinus laevis*) once a week. Of the 12 experimental animals only one started to take non-living food during the first week of testing, whereas the remaining 11 animals stared to eat cod muscle and liver during a period of six months. The walleye that were feeding on cod muscle did not respond to chemical stimulation yet (Fig. 7A). Later the walleye were exposed for 90 second to cod water (supernatant of the water containing minced cod muscle) before receiving a food reward. After seven conditioning trials (Fig. 7B) they were tested again for the responsiveness to cod water, shrimp water and L-alanine. The conditioned responsiveness to the cod water was significantly larger than the responsiveness to shrimp water and L-alanine (Fig. 7C). In the next step the walleye were conditioned to respond to 3×10^{-6} M L-alanine. The responsiveness to L-alanine increased very rapidly to a median of 25 turns of the fish during 90 seconds (Fig. 7 D). These animals responded significantly less (Wilcoxon test; $P<0.01$) to the other tested amino acids, L-leucine, L-arginine, L-proline and L-isoleucine, than to the conditioned stimulus L-alanine (Fig. 6E).

For walleye electrophysiological data indicate functional chemosensory organs (Caprio unpublished), however, the behavioral data conclusively show that walleye that were not conditioned to eat non-living foods, neither respond to amino acids nor use their chemosensory organs in food detection and finding. The extended period of time needed to train fingerlings of walleye to eat non-living foods indicates that the brain networks needed to associate olfactory stimuli with feeding behavior are not fully developed. To express olfactory control of feeding behavior walleye must be conditioned to eat non-living food early during ontogeny.

To initiate sea bass (*Morone labrax*) to eat industrial food pellets a similar early learning procedure is routinely applied in mariculture. At the age of one month the sea bass are, in addition to living rotifers and brine shrimp, offered dried krill and industrial starter feeds (Lewis, 1981; Barahone-Fernandez and Metailler, 1984). These fish can later be raised on industrial food pellets to the commercial size.

DISCUSSION AND CONCLUSIONS

Behavioral experiments provided an important answer to the puzzle: "Why have two chemosensory systems, taste and olfaction, which detect the same water-soluble compounds, evolved in fishes?" Both chemosensory systems are extremely sensitive to amino acids. The channel catfish taste system is even more sensitive to L-alanine than its olfactory system (Caprio 1978). In catfish and cyprinids both systems enable feeding excitatory state that release feeding behavior, however, there are two major differences between the two systems. First, olfaction is broadly tuned, i.e., it enables detection of hundreds and possibly thousand of odors, whereas taste is narrowly tuned, i.e., some fish species detect very few substances with their taste system. And second, olfaction enables learning and odor discrimination, whereas taste does not support learning and discrimination of chemical stimuli.

The need for rapid behavioral responses led to the preservation of the reflex design of the taste-guided turning and snapping/biting behaviors. The rapid reflex networks are situated in the facial and vagal lobes of the medulla, the vertebrate organ that controls the reflex responses. On the other hand, olfactory receptor cells send their axons into the olfactory bulb, which, in combination with the telencephalon, provides the basis for the discrimination of thousands of chemicals. Processing in the brain requires time – the incubation periods for odor perception might extend to more than one second. The odor discrimination process is slow, however; the conditioning to odor stimuli enables the fish to respond most intensely to the olfactory stimuli that foretell the food reward. Odor discrimination prevents loss of energy during aimless search for unrewarded stimuli and even more importantly, unhindered swimming activity of fish during the search for food exposes cryptic fish to predators. By responding less to non-conditioned than to conditioned olfactory stimuli the duration of the predator exposure is shorter than in the case of indiscriminate responses to all olfactory stimuli. Shorter predation exposure times created very strong selective pressure towards the evolution of separate olfactory and taste systems.

NICHE DEPENDENT EXPRESSION OF CONTROL MECHANISMS FOR ESCAPE AND FEEDING BEHAVIOR

To prevent the simultaneous occurrence of conflicting behavioral patterns, escape behavior usually inhibits occurrence of feeding behavior. Even in the closely related bullhead and channel catfish we found large differences in the kinds of stimuli and sensory systems that enable danger detection. Although both species occupy similar ecological niches, danger detection and detection of food depends on entirely different sensory systems. The bullhead catfish

detects danger by low frequency vibrations and food by taste and olfactory stimuli, whereas channel catfish detect danger using visual information and food using visual and chemical information. Food location by the passive electric sense has also been reported for the bullhead catfish (Peters et al. 1974).

The fishes that have been studied to date occupy the following ecological niches, depending on the mechanisms that they use to detect and collect food.

1. *Exclusive chemosensory feeders,* such as black (*Ameiurus melas*) and brown (*Ameiurus nebulosus*) bullhead catfishes have rather inefficient vision. Low frequency vibrations of 0.1-10Hz release fear and escape behavior in the black bullhead catfish. During the long lasting escape excitatory state feeding behavior is totally inhibited. In the absence of vibrations the three chemosensory inputs release behavior as follows:

   (a) Ample olfactory inputs into the thalamo-hypothalamic areas of the brain control feeding excitation. Learning of olfactory stimuli enables perfect olfactory discrimination. With the exception of the pairs L-valine/L-isoleucine and L-alanine/L-serine (and glycine), bullhead catfish discriminate any amino acid from every other amino acid. Catfish initially detect binary and ternary mixtures of amino acids as the more stimulatory components [determined by the amplitude of EOG] in the mixture. Additional discrimination training enables catfish to detect difference between the binary or ternary mixture and its most stimulatory components alone. Bullhead catfish can detect an absence of a single equally effective component within a multimixture of 7 components, however, they cannot detect an absence of a single component in a multimixture of 13 components and they can detect an absence of two or more components in the same multimixture of 13 components.

   (b) The facial taste input enables turning and snapping/biting reflexes and provides large inputs to the thalamo/hypothalamic area of diencephalon to enhance feeding excitatory state. The taste triggered feeding excitation does not allow discrimination of amino acid stimuli. The main snapping/biting stimuli are L-alanine and L-proline.

   (c) The vagal lobe controls swallowing and regurgitation. In bullhead catfish chemical stimuli do not release mastication.

2. *Chemosensory feeders with visual control of fear and escape behavior.* Feeding excitatory states in the channel catfish, *Ictalurus punctatus* are released by visual, olfactory and taste stimuli. Sights of food guide channel catfish directly to the food location. In transparent waters white or lightly colored bottoms inhibit activity and totally incapacitate feeding behavior of channel catfish by daytime. Channel catfish might die of starvation if food is not offered in total darkness, in murky waters or over a dark bottom (Valentinčič and Caprio, 1994). In addition, objects that hide the views of the sky release escape dashes and long lasting freezing responses. Channel catfish also use olfaction and taste in food finding; however, food location with their excellent sight has a priority over its chemical detection. The three chemosensory systems perform the following functions:

(a) Olfaction controls feeding excitatory state and enables learning and discrimination of olfactory stimuli.

(b) The facial taste system enables turning and snapping/biting reflexes and also controls the feeding excitatory state. L-alanine, L-proline and L-arginine are the main snapping/biting stimuli. The facial taste functions are essentially the same as in bullhead catfish except for L-arginine sensitivity that is high in the channel catfish and absent in the bullhead catfish.

(c) The vagal taste system controls mastication, swallowing and regurgitation. High concentrations of L-arginine above ten millimole release mastication.

3. *Omnivorous cyprinid fishes* such as carp (*Cyprinus carpio*) and goldfish (*Carrasius auratus*) use vision, olfaction and taste in food finding. Carps are fearless fish, their escape behavior is controlled mostly by visual and mechanical stimuli that release escape dashes. Unlike in catfish long-term activity inhibition was not observed in this group of fishes. Carps feed after visual detection of food, however, in murky waters they rely upon olfaction and taste to excite, locate and snap at food. Their well developed olfactory and taste systems participate in the control of feeding excitation, snapping/biting, intra-oral food evaluation and food sorting behavior. The three chemosensory systems enable the following functions.

(a) The olfactory system enables control of feeding excitation and olfactory discrimination (Zippel et al., 1993; Von Rekowski and Zippel, 1993).

(b) The facial taste system triggers snapping/biting reflexes; L-proline and L-alanine are the main snapping/biting stimuli (Valentinčič, unpublished).

(c) The vagal taste system performs food-sorting behavior (Finger and Kanwal, 1992; Lamb and Finger, 1995).

4. *Visual feeders that depend on olfaction to increase feeding excitatory state.* Farm-raised rainbow trout, *Oncorhyncus mykiss*, use vision or olfaction to find food. The rainbow trout are fearless fish, if no damage is done during netting and manipulation of rainbow trout the escape excitatory state wanes within 1-3 days compared to 1-3 month in the catfish. Rainbow trout can be conditioned quickly to feed after detection of any visual, vibrational and chemical stimuli associated with food. Farm raised rainbow trout respond to olfactory stimuli and even feed frenzy stimulated by amino acids alone. Their chemosensory systems performs the following functions:

(a) Olfaction enables feeding excitation and learning amino acid stimuli.

(b) At a post alevin stage of life the facial taste system does not control snapping/biting reflexes. Snapping biting responses to taste stimuli were observed only at the pre-feeding alevin stage. On the other hand the snapping/biting behavior of adult rainbow trout occurs exclusively under the visual control, it is an integral part of the complex feeding behavior.

(c) The vagal taste system serves oral for food evaluation and yields food reward information (Valentinčič and Caprio, 1997). Cotton food models soaked in amino acid

solutions were retained longer in the mouth than the water soaked test model indi-
cating that amino acids enable intraoral food evaluation that eventually leads to
swallowing or rejection (Jones, 1989). L-proline and L-leucine were the most
effective stimuli for the oral retention of the cotton food models.

5. *Exclusive visual feeders that were not conditioned to eat non-living foods during their early life
do not use olfactory and taste systems to control feeding behavior.* European huchen (*Salmo
hucho*. Fam. *Salmonidae*) and walleye (*Stizostedion vitreum*, Fam. *Centrachidae*) occupy the
exclusive visual hunters' niche. Electrophysiological data indicate that the walleye
olfactory system is broadly tuned and its taste system is very narrowly tuned. On the
other hand behavioral data for wild huchen and walleye showed that over 90% of the
fish captured in nature do not use chemical senses to release feeding excitatory state,
they neither locate nor pick up food with chemical senses. Food location, capturing prey
and even feeding frenzy depend exclusively on vision. There is strong evidence that
snapping/biting behavior is under visual control. In addition to chemical senses the
intra-oral food evaluation depends on surface quality and softness of the lure. The
predatory fishes sometimes swallow "living boxes", such as hard-shelled crayfish that,
unless broken by the predators, nearly do not leak dissolved amino acids.

Early experience modifies the behavior of walleye, huchen and sea bass. After experience
with non-living food these fish are excited by olfactory stimuli alone and can be conditioned to
amino acids. We transferred fingerlings of walleye from living minnows to fish muscle and to
industrial food pellets. Predatory fish that started to eat non-living foods also started to use their
olfactory systems to maintain feeding excitation and discriminate amino acid stimuli.

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Olfactory Imprinting in Salmon: New Models and Approaches[1]

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ABSTRACT

Olfactory imprinting is a specialized form of unconditioned learning in which olfactory information is acquired and then used in some specific behavioral context later in life. A key characteristics of an imprinted memory is that it is formed during a sensitive period of development. This prerequisite thus distinguishes olfactory imprinting from other types of odor learning in which only conditioned exposure to an odor stimulus is required for learning to occur. Most investigations designed to explore the mechanisms underlying olfactory imprinting have focused on mammalian species, concentrating on synaptic events at the level of the main and accessory olfactory bulbs (Hudson 1993). Recent integrative studies with salmon (Dittman et al. 1997; Nevitt et al. 1994) and rabbits (Semke et al. 1995), however, provide compelling evidence that highly specific imprinted odor memories may also be retained in the periphery, i.e., at the level of the olfactory epithelium proper. These results suggest that populations of olfactory receptor neurons may be selectively tuned to respond to odor molecules present during a hormonally linked sensitive period. A potential key to the mechanism of how these peripheral odor memories become established draws on the unique ability of olfactory receptor neurons to turn over throughout an organism's life span (Farbman 1994). How hormonal and environmental factors work together to influence olfactory neurogenesis is not yet been rigorously addressed in the literature (Shepherd 1994), but ultimately may provide important new insights not only for basic science but for salmon conservation as well.

Key words: Salmon, Olfactory Imprinting, *Oncorhynchus kisutch*, Memory Formation

INTRODUCTION

To the cellular neurobiologist who feels at home in a pair of hip waders, a coho salmon (*Oncorhynchus kisutch*) presents a tractable model species for examining mechanisms of

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olfactory imprinting and memory fundamental to other vertebrates. Even a child in grade school can appreciate that a fiery salmon leaping up massive waterfalls through grizzly bear-infested waters presents a certain olfactory charisma. But in the laboratory these animals also offer many useful advantages for studying basic mechanisms of olfaction. For starters they have an acute sense of smell. Indeed, almost every aspect of their lives is influenced to some degree by olfaction (including feeding, predator avoidance, reproduction, and migration) and the under-lying endocrine factors that might influence their olfactory behaviors have been well worked out. Their ability to imprint and home to controllable olfactory cues learned during a sensitive period of development called the parr-smolt transformation (smolting) offers a great experimental system to study these basic questions (Hasler and Scholz 1983).In coho salmon, smolting coincides with surges in plasma thyroid hormone levels that are believed to be impor-tant for olfactory imprinting, as well as many other physiological and behavioral changes that occur at that time (Folmar and Dickhoff 1980; Hoar 1988). Since plasma thyroid hormone levels are easily manipulated, it is possible to determine their effects on both specific neural structures and neurophysiological changes involved in imprinting.

We began investigating this problem as graduate students at the University of Washington in the mid-1980s. Our studies were unique in that we used a wide array of techniques ranging from fine-scale electrical recording of isolated olfactory receptor neurons and biochemistry to extensive field behavioral studies. Our aim was to explore the underlying mechanisms that produced a memory for the home stream. The picture that has emerged from this combined effort suggests that olfactory imprinting involves a tuning of olfactory receptor cells to specific stream odorants, and that this tuning is hormonally driven. A key element of this model is that a proliferation and selective survival of olfactory receptor neurons in the periphery drives olfactory imprinting in the brain, or specifically, in the olfactory bulbs where primary processing occurs. This novel approach to olfactory imprinting links olfactory neurogenesis to a well established, olfactory mediated behavior requiring both learning and memory. This paper reviews the conceptual framework that has lead us to this new model.

What we Knew About the Mechanism of Salmon Imprinting

Pacific salmon have long been recognized for their long distance homing migrations which bring them back to their natal spawning grounds to reproduce and so complete their dramatic life cycle (Fig.1). Numerous field and laboratory experiments have demonstrated an olfactory basis for this remarkable behavior: at a critical period of development, juvenile salmon imprint to the odorant signature of the home stream. Years later, mature adults use this olfactory memory to guide them home (Quinn and Dittman 1990). In a classic demonstration of olfactory imprinting (Scholz et al. 1976), Hasler and colleagues showed that salmon could be manipulated to return to an arbitrary stream scented with particular synthetic chemicals such as morpholine (MOR) or phenyl ethyl alcohol (PEA), provided that these fish were first briefly exposed to these odors during parr-smolt transformation several years earlier. Without this priming, fish showed no behavioral response to either odorant (Hasler and Scholz 1983; Scholz et al. 1978; Scholz et al. 1976; Sutterlin and Gray 1973). This sensitive period, also referred to
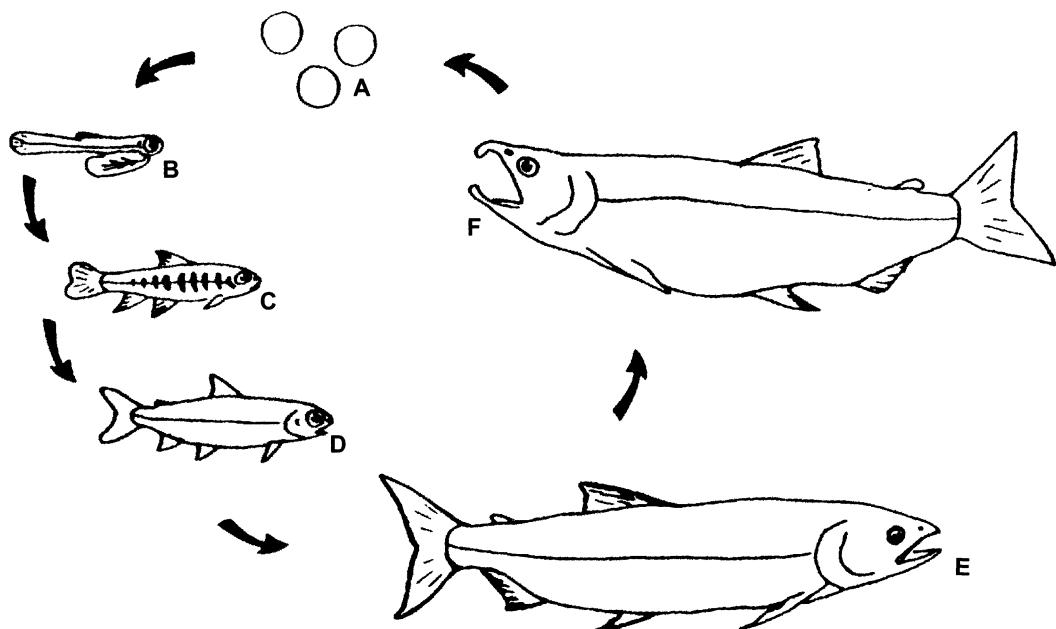
Fig. 1 Life history of coho salmon: A. Eggs are laid each fall and hatch in freshwater streams. B. Hatchlings (alevins) reside in the gravel absorbing nutrients from their yolk sac. C. Parr continue to live and grow in freshwater streams until the following spring when they undergo the parr-smolt transformation which allows them to live in salt water. D. Smolts migrate downstream to begin life in the open ocean as sea-run salmon E. Two to three years later, mature spawning salmon (F) return to their natal streams to spawn (From Nevitt and Moody 1992).

as "smolting", is a transitional phase somewhat analogous to amphibian metamorphosis (Folmar and Dickhoff 1980). Smolting is associated with surges in plasma thyroid hormones, and is characterized by a suite of physiological and behavioral changes that prepare young stream-dwelling salmon parr for life in the open ocean (Fig. 2). These changes include an increase in gill Na^+/K^+ ATPase activity, a silvering of the body, a shift in rheotactic orientation, and the ability to tolerate salt water (Hoar 1988).

Since olfactory imprinting has been linked to smolting, it has been hypothesized that surges in thyroid hormones experienced during this time help to establish the imprinted olfactory memory (Hasler and Scholz 1983; Scholz 1980). Coho salmon fail to home to artificial odorants experienced at early life stages when plasma thyroid hormone levels (T_3 and T_4) are comparatively low. However, artificially elevating T_4 to smolting levels stimulates precocial imprinting (Scholz 1980). Similarly, it has been reported that Atlantic salmon (*Salmo salar*) have an optimal period for long-term olfactory learning which coincides with peak levels of thyroid activity (Morin et al. 1989), though it has also been shown that olfactory imprinting in coho can occur in the absence of a dramatic plasma T_4 surge (Dittman et al. 1994). Since it has been established that T_4 is enzymatically converted extrathryoidally to the intracellularly active form T_3 (DeGroot et al. 1978; Higgs et al. 1982) it is tempting to hypothesize that T_3 acts on the neural
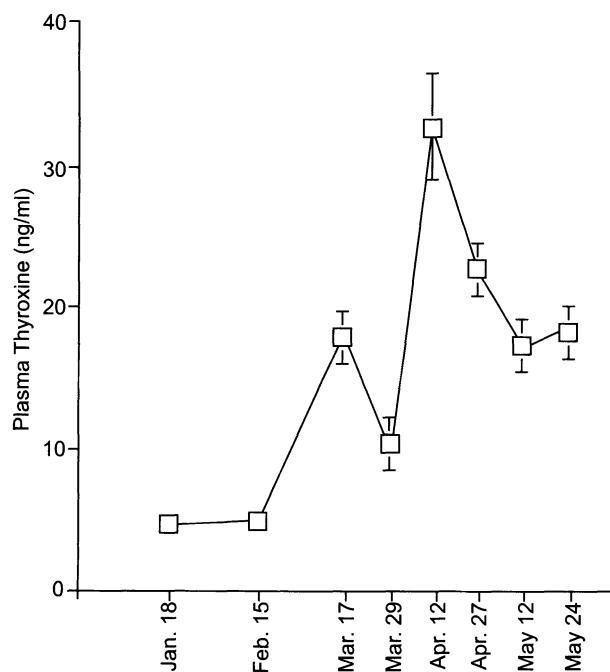
Fig. 2 (A) Coho salmon *(Oncorhynchus kisutch)* before and after the parr-smolt transformation. Parr-stage fish are characterized by smaller body size and vertical stripes (parr marks). In contrast, smolts (upper fish) are larger and highly silvered. (B) Accompanying surge in plasma thyroid hormone levels across the parr-smolt transformation (Nevitt and Jarrard, unpubl.).

substrate for imprinting. Direct neural effects of these hormones have been widely documented in many vertebrates. For example, thyroid hormones (T_3 and T_4) promote cytoarchitectural changes, including increases in dendritic arborization of neurons (Denver 1998, Hoskins and Grobstein 1985, Rami et al. 1986a, Rami et al. 1986b), synaptogenesis in the CNS (Gould and Butcher 1989; Hofmann et al. 1989), increased functional expression of specific membrane receptors (Brent et al. 1991, Glass and Holloway 1990, Oppenheimer et al. 1987, Sammuels et al. 1989) and neurogenesis in peripheral olfactory systems of other vertebrates (Burd 1990; Burd 1991; Mackay-Sim and Beard 1987; Meisami et al. 1990; Paternostro and Meisami 1994; Paternostro and Meisami 1996).

Although results from experimental manipulations point to smolting and the accompanying surge in thyroid hormone as the only sensitive period for imprinting, this now traditionally accepted model is not borne out by natural movement patterns of young fish within river systems. Upon hatching, most species of juvenile salmon leave their incubation sites within weeks of hatching, often smolting miles from their place of birth. For example, it is well established that one of the most faithful homers, Sockeye salmon (*O. nerka*) typically spawn in streams and migrate to nursery lakes where they eventually smolt one to two years later. Yet these fish return to spawn in their natal streams rather than in the lakes (Varnavskaya et al. 1994; Wood 1995). Even species with a relatively "simple" life history pattern like coho salmon, which imprint as smolts when reared in the hatchery, demonstrate migration patterns in the wild that suggest they must imprint prior to smolting. For example, coho salmon often make extensive migrations downstream from their natal stream in the winter prior to their parr-smolt transformation (Peterson 1982), yet home as adults to their natal site. The implication is that in natural situations, sensitive periods for imprinting may be more plastic than for fish reared in the relatively monotonous environment of the hatchery.

Behavioral Studies Confirm the Parr-smolt Transformation is the Sensitive Period for Imprinting for Hatchery-reared Fish

In light of these discrepancies, our first experiments were designed to challenge the idea that the parr-smolt transformation was the only sensitive period for imprinting. Morphological and physiological evidence suggested that the salmonid olfactory system was functional as early as hatching (Hara and Zielinski 1989), and that soon after emergence, salmon were able to learn odors associated with specific habitats and odors from other fishes (Dittman and Quinn 1996; Quinn and Dittman 1990). Furthermore, working at the University of British Columbia, Simon Courtenay (Courtenay 1989) had demonstrated that juvenile coho salmon exposed to a synthetic odorant (morpholine) shortly after or even *before* hatching, were able to learn and retain a memory of this odorant over a year later. If these odor memories established early on were also used for homing, this finding would suggest that plasticity in neuronal development early on in the fish's life might play a role in learning the scent of the home stream.

Our first investigations examined the timing of olfactory imprinting by exposing juvenile coho salmon to either natural stream waters or to an artificial odorant (PEA) at three specific developmental stages (as alevin, parr and smolting fish). We chose this rosy smelling odor

because Hasler had used it in his classic studies of salmon imprinting (Scholz et al. 1976). We then reared the fish to maturity and tested their behavioral responses. Our experiments involved presenting "homing" fish with natural choice experiments between tributaries scented with PEA and control streams. These experiments were conducted on the same site (Issaquah Creek, Washington) where forty years earlier Hasler and his student Warren Wisby had conducted their original experiments demonstrating that olfaction was required for homing (Dittman et al. 1996; Hasler and Wisby 1951). In a second behavioral test, we also placed fish downstream of a divided spawning channel, scenting one side with PEA at concentrations (100 nM) used for imprinting. In these separate experiments, only salmon exposed to PEA specifically during the parr-smolt transformation demonstrated an increased behavioral attraction to this odorant as adults (Fig. 3) (Dittman et al. 1996). We found no significant evidence that this species became imprinted to homing odors prior to this developmental stage. These experiments confirmed that the parr-smolt transformation was the sensitive period for imprinting at least in our hatchery-reared salmon. The challenge would be to come up with a physiological model that would elucidate both the patterns of homing noted for wild fish as well as the experimental data suggesting that the timing of imprinting was restricted to the parr-smolt transformation.

Once we had established that smolting was the sensitive period for imprinting for our hatchery-reared fish, the next part of our investigations aimed to tease apart some simple aspects of the underlying mechanisms contributing to this memory. At this time, most people
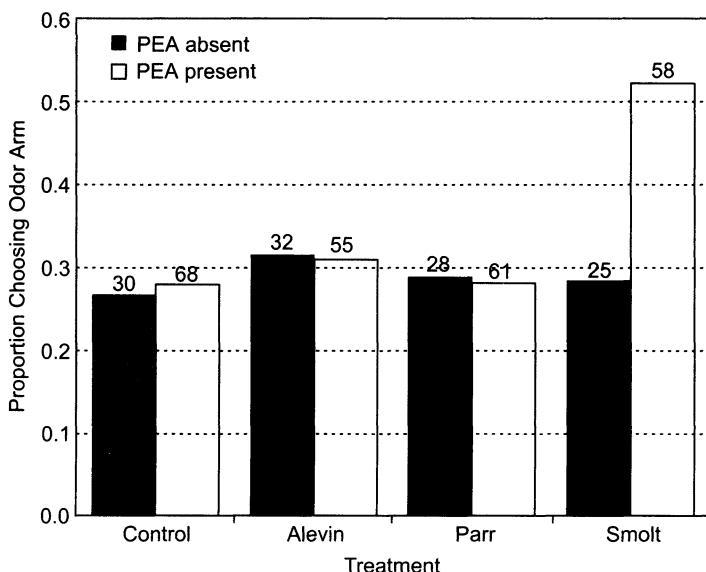
Fig. 3   Behavioral responses of mature coho salmon to PEA in the Big Beef Creek divided spawning channel. Salmon were exposed to PEA at the developmental stage indicated or never experienced PEA (control). Open bars show the proportion of salmon choosing arm B before PEA was added. Shaded bars show the proportion of salmon choosing arm B in the presence of PEA metered into arm B. Numbers above bars indicate the total number of fish choosing either arm A or B (From Dittman et al. 1996).

working in the field believed that olfactory memories were born centrally, i.e., in the brain, but efforts to pinpoint neural correlates of imprinted memories in salmon were largely inconclusive. Results from independent studies had reported gross changes in electroence-phalographic (EEG) activity in imprinted salmon in response to homestream waters (Hara et al. 1965; Ueda et al. 1967). Investigators attributed these electrical responses to odor-induced fluctuations in activity in the olfactory bulb, the primary processing center for incoming olfactory information, and speculated that some aspect of the imprinted memory must be stored there and later retrieved. Despite these initial findings, debate in the literature continued for years because these studies could not be reliably repeated (Cooper and Hasler 1974; Cooper and Hasler 1976; Dodson and Bitterman 1989; Hara and Brown 1979; Hara et al. 1984; Morin and Doving 1992; Ueda et al. 1967). Amidst the controversy that ensued, no testable model emerged that implicated a mechanism for how odor memories might be formed.

The beauty of working at the University of Washington's school of fisheries is that salmon are part of the culture there. Each fall runs of both Chinook (*O. tshawytscha*) and coho salmon home literally to the back door of the school where they provide a dramatically tangible inspiration for a person engaged in a problem like olfactory imprinting-something that a lab rat might not so easily inspire. We thought that a clue to understanding this problem might be in stepping back far enough to recognize what activity in the olfactory bulb might imply. This change of perspective brought us back to the nose where specialized cells called olfactory receptor neurons detect odorants. In salmon as in other fishes, olfactory receptor neurons are embedded in a mucous coated ciliated epithelium, which is folded like a flower into a rosette arrangement. The folding increases the surface area available for receptor cells to detect odors. Look four hundred times closer and olfactory receptor neurons resemble elongated bowling pins with cilia on the top end and a long axon streaming from the base. The cilia are enriched with receptors that bind odors, triggering a cascade of biochemical reactions that transduce this odor signal into an electrical response that is, in turn, transmitted centrally to the brain. In a mature salmon, axons arising from a $10\,\mu m$ cell can be more than a centimeter long, terminating in the glomerular layer of the olfactory bulb of the brain. This new perspective gave us a fresh way to think about a mechanism: if the olfactory bulb did serve as a substrate for olfactory memory, then perhaps part of that substrate was in the olfactory receptor neurons themselves since these neurons contributed to some of that activity.

Our initial experiments were thus aimed at characterizing the electrical properties of ciliated olfactory receptor neurons isolated from coho salmon using a fine-scale electrical recording method called the patch clamp technique (Nevitt 1990; Nevitt and Moody 1992). These efforts showed that ciliated olfactory receptor neurons isolated from salmon had broadly similar ionic conductances to olfactory receptor neurons that have been studied in other organisms. However, these results also showed significant variations in electrical properties linked to life-stage differences. In cells isolated from pre-smolts, a Ca^{2+} - dependent K^+ current dominated the outward current, whereas in cells isolated from smolted fish, a transient K^+ current became prominent (Fig. 4). We also identified and described consistent differences in the response characteristics of olfactory receptor neurons to internal dialysis with second messengers (Nevitt and
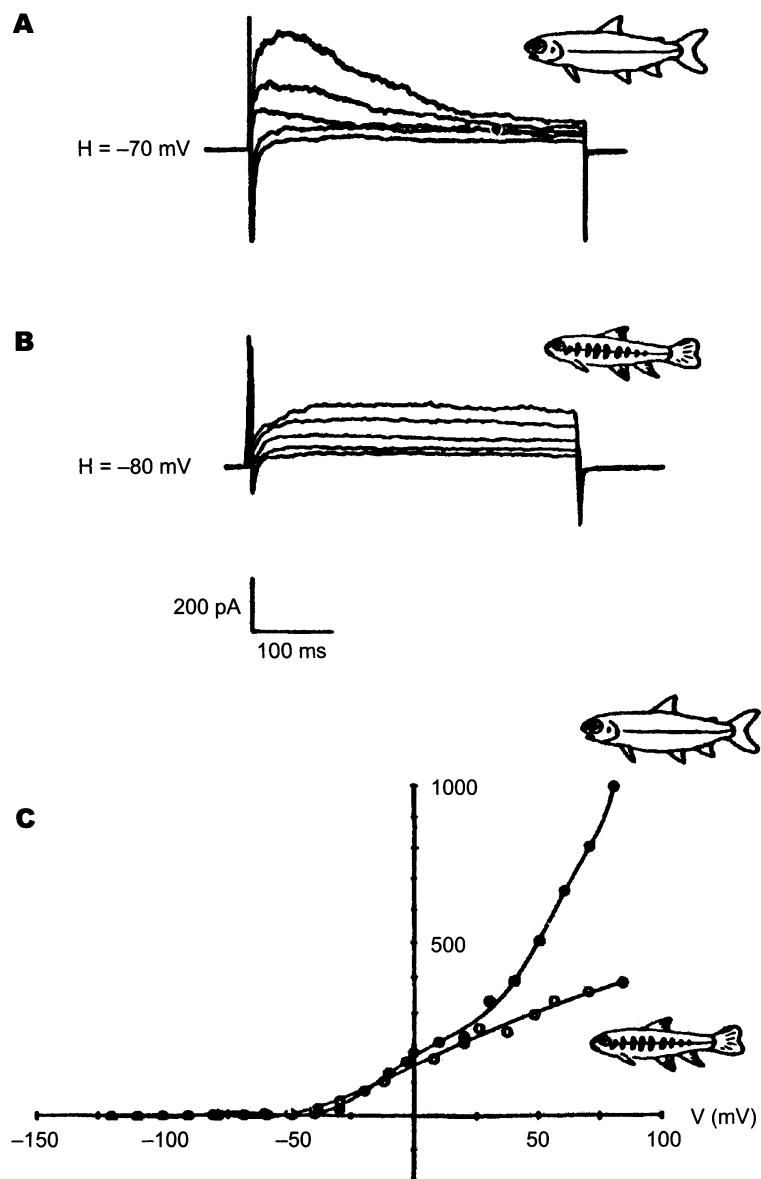
Fig. 4   Outward currents: life-stage differences. Families of current traces recorded from cells isolated from a smolt (A) and a parr (B). External Sr^{2+} was used to block the Ca^{2+}-activated K^+ current. Steps are to -23, -12, +8, +26 and +56 mV and to -20, -10, +20, +40 and +60 mV respectively. C. The corresponding peak current-voltage relationship for the families of current traces in A (o) and B (□). (From Nevitt and Moody 1992).

Moody 1992). Together these data implied that olfactory receptor neurons were far from static detectors of odors in the environment, and this piqued our curiosity to examine the idea that the peripheral olfactory system might contribute to homestream learning.

The first step in this challenge was to hand rear ten thousand coho salmon through the parr-smolt transformation. Luckily we were already doing this in conjunction with our behavioral experiments discussed above – in fact these mechanistic studies were planned to complement our behavioral investigations. Rearing our own animals also gave us control over the olfactory environment that these fish experienced during their growth and development. Upon smolting, we exposed an experimental group of fish to PEA (100 nM) for ten days while a second, unexposed group served as controls. Fish were then coded by fin clipping and reared together in a common facility. The following fall and winter, we measured PEA responses in isolated ciliated olfactory receptor cells using patch clamp recording techniques in double-blind trials. We found that olfactory receptor cells isolated from PEA-imprinted fish were nearly twice as likely to respond to PEA compared to those isolated from naïve fish of the same cohort (Fig. 5) (Nevitt 1990). Furthermore, we found that cells from imprinted salmon showed a six-fold increase in responsiveness to PEA compared to cells from naive fish of the same cohort. Cells isolated from both PEA-imprinted and naïve fish responded similarly to L-serine, a different odorant that salmon can smell, suggesting that the change in sensitivity was specific to the imprinted odorant.

The results of these experiments suggested to us that some component of the homestream memory was encoded in the peripheral olfactory receptor cells themselves. In characterizing the electrical properties of isolated olfactory receptor neurons, we had noted differences associated with the timing of cell harvesting with respect to the salmon life cycle. These preliminary results suggested that second messengers (specifically cGMP) might play a role in modulating odor-induced electrical excitability of olfactory receptor neurons, but that this effect was linked to the fish's developmental state (Breer et al. 1992; Nevitt and Moody 1992; Restrepo et al. 1993).

However, while cAMP had also been implicated as a major signaling molecule in other vertebrates (Chen et al. 1986; Sklar et al. 1986), our early results suggested that the imprinting odorant PEA had little effect on pathway regardless of the fish's developmental state (Dittman 1994; Dittman et al. 1997). Together these results led us to hypothesize that imprinted odors might have different effects on different second messengers and provided a different mechanism through which to explore our hypothesis that peripheral imprinting contributed to homestream learning.

Because cGMP is produced by the activation of guanylyl cyclase activity, our next set of experiments focused on how odorant exposure effected the activity of this enzyme in isolated olfactory cilia. Specifically, we examined guanylyl cyclase activity in the presence of PEA in olfactory cilia isolated from PEA-imprinted and non-imprinted salmon - members of the same cohort on which both behavioral and electrophysiological experiments had been performed. Behavioral sensitivity to imprinted odorants had been linked to maturational state in other studies, so we conducted our experiments using cilia isolated eight months prior to maturation and also periodically during the period of final maturation and spawning. We found that stimu-
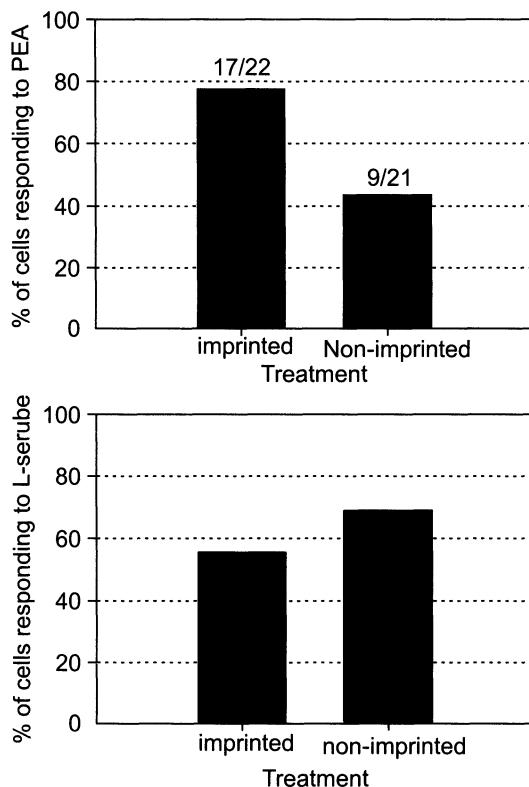
Fig. 5 Responses of isolated olfactory receptor neurons to PEA and L-serine. A. Cells isolated from PEA-imprinted and PEA-naïve fish were scored as positive or null responders to PEA in (10^{-6}-10^{-8} M) in double-blind trials. Percentages of cells responding to PEA were significantly different between the two groups of cells (17/22 PEA-imprinted; 9/21 naïve; $p<0.05$ G-test). B. Percentages of cells responding to a second odorant, L-serine (10^{-5}-10^{-8} M), were not significantly different between the two groups (10/18 PEA-imprinted 13/19 naïve; $p>0.05$, G-test).

lation of guanylyl cyclase activity by PEA was significantly greater in olfactory cilia isolated from PEA-imprinted salmon compared with PEA-naive fish only at the time of the homing migration, two years after PEA exposure (Fig. 6) (Dittman et al. 1997). These results suggested that sensitization of olfactory guanylyl cyclase may play an important role in olfactory imprinting.

Altogether, our results suggested to us that exposing salmon smolts to nanomolar concentrations of PEA for as little as 10 days could be correlated with dramatic and measurable changes in their peripheral sensitivity to odors even years later. But how could we reconcile this data into a workable model, particularly since olfactory receptor neurons were thought to turnover throughout the fish's life? Since this sensitive period for odor exposure was linked to surges in plasma thyroid hormone levels, we guessed that the changes in peripheral sensitivity we had discovered might also involve a hormonally-driven modulation in the expression of particular
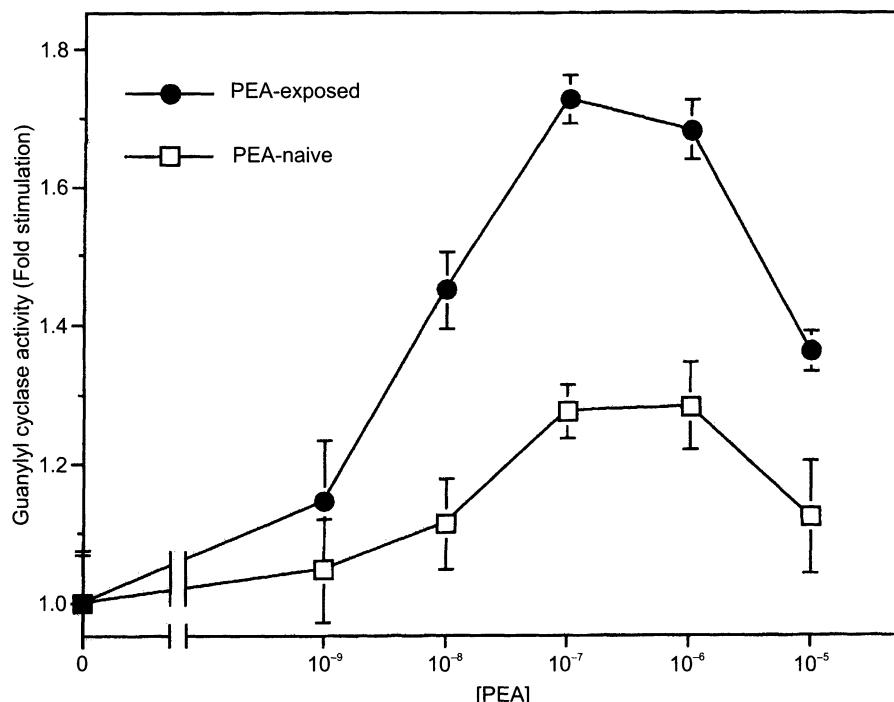
Fig. 6 Sensitivity of guanylyl cyclase to PEA in olfactory cilia isolated from PEA-exposed and PEA-naive salmon. Cilia were prepared from 10 PEA-exposed or PEA-naive salmon. Brain membranes were prepared from PEA-exposed fish. Cilia were assayed for guanylyl cyclase activity in the presence of 2.5 mM $MnCl_2$, 0.5% Lubrol PX, and PEA. (From Dittman et al. 1997).

olfactory receptor proteins. Populations of receptor neurons that expressed these proteins might also be affected (Buck and Axel 1991; Ngai et al. 1993; Shepherd 1994). Either idea was consistent with differences in outward current components in olfactory receptor neurons isolated before and after smolting that we had reported. Moreover, other reports were indicating that the olfactory epithelium of smolting salmon (O. masou) was enriched in thyroid hormone receptors compared with epithelium from parr (Shimizu et al. 1995). These data offered further support to a model invoking thyroid hormone as a modulator in this system. At the same time, additional discoveries of odorant-induced peripheral plasticity in rabbits (Semke et al. 1995) and olfactory-deficient strains of mice (Wang et al. 1993) using electro-olfactogram (EOG) recording techniques suggested that our results might have broader implications for environmentally-induced plasticity in the olfactory epithelium in vertebrates.

Other clues came from scattered studies investigating changes in olfactory neuroanatomy across the parr-smolt transformation. Unpublished work with Atlantic salmon (Salmo salar) suggested a quadrupling of olfactory receptor cell number, as well as specific changes in the relative composition of the olfactory bulb neuropil during this transition (Bowers 1988). Similar studies with Chinook salmon later confirmed these findings (Jarrard 1997). These data

implied that populations of olfactory receptor neurons were proliferating specifically during the parr-smolt transformation, though detailed studies relating hormones to proliferation had not been done. More precise anatomical studies showed the primary olfactory projection patterns in the glomerular layer of the olfactory bulb for a salmonid (Rainbow trout: *O. mykiss*). Working at the University of Michigan, David Riddle and Bruce Oakley had recently identified nine distinct terminal olfactory receptor cell projection fields ranging in size from 1% to 35% of the glomerular layer. This was the area where olfactory receptor neurons form synapses in the brain, but the functional relevance of this segregation was unclear (Riddle and Oakley 1992).

A Working Model of Olfactory Imprinting

Based on our own data and the information available to us from other studies we formally proposed a new model for olfactory imprinting in salmon (Nevitt 1995). Our model suggests that:

(1) During sensitive periods for imprinting, thyroid hormones (T_3 and T_4) promote a non-specific proliferation of olfactory receptor neurons that are sensitive to a wide variety of odors.

(2) Receptor cells that are most active (i.e., responsive to the odorants present in the environment) survive, while others die. Selective survival may involve competition for synaptic targets.

(3) This punctuated proliferation and selective survival of olfactory receptor cells triggers a reorganization of glomerular structures within the bulb.

After publishing our first paper suggesting our new model for olfactory imprinting (Nevitt et al. 1994), we were pleasantly surprised to receive a letter from Robin Hudson at the Institut für Medizinische Psychologie in Munich, Germany. Hudson also studied olfactory imprinting, but in a different species altogether: the European rabbit (*Oryctolagus cuniculus*). Hudson and co-workers (Semke et al. 1995) had observed that if pregnant mothers were fed aromatic juniper berries (part of the their diet in nature), then at weaning time, their pups preferred to eat juniper, even if they were reared by a foster mother fed standard laboratory rabbit chow. More-over, this learning event was accompanied by a dramatic proliferation of olfactory receptor cells that occurred post-natally when pups were suckling. Her results also showed an enhanced sensitivity of the pup's peripheral olfactory system to juniper, but only if the mother had eaten juniper while pregnant. The implication was that the young pups' noses were tuned to be super sensitive to odors that were associated with the food that their mother ate, and that this tuning was reflected in neural proliferation and changes in sensitivity in the peripheral olfactory system. Working independently, in parallel and on phylogenetically different systems, we had come up with nearly identical models for peripheral olfactory imprinting.

Implications of the Model

This model suggests that populations of olfactory receptor neurons may be selectively tuned to respond to odor molecules present during a hormonally linked sensitive period. The

evidence we have reviewed suggests that salmon imprint to homestream odors at the parr-smolt transformation when thyroid hormones surge. However, more subtle peaks occur much earlier in development, particularly at hatching when fish emerge from their natal gravel (Dickhoff and Sullivan 1987; Tilson et al. 1994; Tilson et al. 1995).Detailed electrophysiological investigations suggest that hatchlings (alevins) respond to a variety of odors (Hara and Zielinski 1989).If the olfactory system is competent to respond to thyroid hormone at these early stages, then changes in levels of this hormone may well contribute to olfactory imprinting.

One of the strengths of this model is that it bridges a gap between results implicating the parr-smolt transformation as the only sensitive period for imprinting and observations of migratory patterns of wild runs that suggest that the timing of imprinting is more flexible. We think that hatchery-reared salmon experience sensitive periods for imprinting predominantly during developmentally controlled times when thyroid hormone surges, or just after release from hatcheries when their environment is rapidly changing (Dittman and Quinn 1996).In hatchery rearing facilities, water quality, temperature, flow rate and diet are all carefully controlled, and housing methods typically eliminate territorial and other behaviors that juvenile salmon would naturally be expressing during their early life history. In contrast, wild salmon may experience a greater plasticity in imprinting because the thyroid-endocrine axis is influenced by the environment that a young fish experiences (Dittman and Quinn 1996). Under more natural conditions, patterns of movement within the river system brings a young wild fish in contact with a myriad of enrichment including different water sources, temperatures and flow rates, and any of these factors can stimulate thyroid hormone production (Dickhoff et al. 1982; Hoffnagle and Rivizzani 1990; Iwamoto 1982; Lin et al. 1985; Youngson and Simpson 1984). Our model thus does not restrict learning to the parr-smolt transformation, but links imprinting events with increases in thyroid hormone. The basic idea is that when thyroid hormone surges and is converted to T_3, neural proliferation and pruning follow, in a sense tuning the fish's peripheral olfactory system to the river system that it has experienced throughout its early life (Fig. 7).

This model also carries with it important considerations for conservation of wild salmon runs, particularly with respect to influences imposed by hatchery rearing. Outside of Alaska, Pacific salmon populations have been declining in both number and diversity for the past 100 years despite multi-million dollar investments in hatcheries to ensure their survival. These declines can be attributed to a number of factors including habitat destruction, over-fishing, dams, agricultural practices and, indeed, even hatcheries themselves (Moyle 1998).Hatchery-reared fish often demonstrate inappropriate behaviors when released in the wild (e.g. increased aggression in feeding, impaired mating behavior, increased levels of straying). Thus, hatchery practices can have profound ecological and genetic consequences on the hatchery population as well as the wild populations with which they may interact (Fleming and Gross 1992; Fleming and Gross 1994; Grant 1997; Moyle 1998). Our growing understanding of the potential for plasticity in imprinting helps us to appreciate that an adult spawning salmon is not simply the product of a genetic stock, but is shaped by the environment in which it is reared. The neural blueprint that is modified through development translates early experience into behaviors
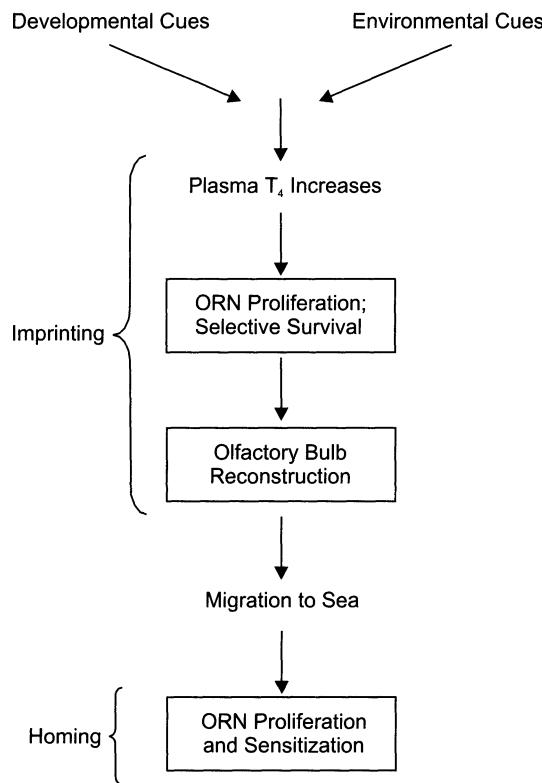
Fig. 7 Olfactory plasticity may be driven by surges in thyroid hormone. This hypothetical model illustrates a possible scenario for incorporating environmental influences on early neural development into our model of olfactory imprinting. (ORN indicates olfactory receptor neuron).

expressed later in life - behaviors that may influence the reproductive success of the individual as well as the population. Conservation efforts that ignore this basic tenant may well produce bodies, but do nothing to preserve the reproductive integrity of a salmon run over the long term if plasticity in the neural and subsequent behavioral development of the animal is not appreciated.

The model we present offers a framework for investigating olfactory imprinting in salmon and possibly other systems as well. The mechanisms involved are bound to be more complex than this simple model suggests, but our aim is to offer a conceptual outlook for future investigation, linking olfactory experience during a sensitive period of development to functional reorganization of the olfactory bulb (Brennan and Keverne 1997). Artificial odors serve as a useful tool for investigating mechanisms of olfactory imprinting, but they do not simulate the natural environment. Instead, they simplify the system so that we can study its components. And in fact, however unrealistic to the natural experiences of wild salmon in natural river systems, the combination of hatchery rearing and controlled enrichment using artificial odorants has lead to substantial improvements in our understanding of the underlying mechanisms contributing to olfactory imprinting.

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Sensory Brain Areas in Deep Sea Slickheads, Eels, and Grenadiers: Comparison of Mesopelagic and Demersal Species

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ABSTRACT

The sensory brain areas of a sample of more than one hundred deep-sea fish species were studied and the relative volumes of the olfactory bulb, optic tectum, octavolateral area, and the gustatory area determined. In the absence of direct observations on the behavior of this ichthyofauna these data allow to make deductions about the kinds of sensory modalities used preferentially in the remote deep-sea environment. In the present chapter, members of three families are compared that have representatives living on or near the sea floor ("demersal") and in the open water between 200 and 1,000 m (mesopelagic). The findings indicate that both regions present fish with rich and diverse sensory environments. While vision emerges as the dominant sense of the mesopelagic realm, olfaction seems more important on or near the bottom of the sea. However other sensory modalities supplement these senses in species-specific patterns. Considerations of the phyletic relationships indicate different degrees of pervasiveness. Whilst in slickheads the dominance of vision appears to be a family related trait, similar common features are found neither in the eels nor in the grenadiers. By contrast, the common trait in these two families seems to be the greater adaptability to the environment.

Key words: Deep-sea fish, Olfactory bulb, Optic tectum, Octavolateral area, Gustatory area

INTRODUCTION

The deep sea comprises about 99.5% of the total volume of biological habitats on earth (Cohen 1994; Angel 1997), and contains the most abundant population of vertebrates. At the limits of sunlight penetration i.e. between 200 and 1000m there is an abundant and diverse community of fish whose lifestyles have adapted to the constraints of pelagic life. This environment is dominated by rapidly declining levels of sunlight, currents and associated regional changes in salinity, temperature and nutrients (Pinet 2000). The ichthyofauna of this mesopelagic

habitat consists mostly of relatively small specimens (several cm up to about 35cm total length) and is surprisingly speciose (509 different species; Merrett und Haedrich 1997). Among the most typical representatives are hatchetfish (*Sternoptyx*), viperfish (*Chauliodus*), the "swimming mouths" (*Eurypharynx*), eels, the well-known anglerfish (*Ceratias*), and *Cyclothone*, as the most abundant vertebrate on earth.

By contrast, the floor of the deep sea is home to a community of fish that have adapted to the special conditions of high hydrostatic pressure, low temperature, total absence of sunlight, and a sparse supply of food. Fishes of the abyss can be divided into a benthic population which inhabits the bottom of the continental slopes, rises, and abyssal plains and secondly, a benthopelagic population roaming the water layers close to the bottom (from 1000m to 6000m; Marshall and Merrett 1977; Pinet 2000). On a gross morphological level, this demersal fish fauna is remarkably different from the mesopelagic community. First of all, most demersal fish are considerably larger than those living at shallower depths in the water column (for a detailed discussion of the "bigger-deeper hypothesis" see Merrett and Haedrich 1997). Furthermore, their coloration (lack of countershading), jaw structure, musculature and fin morphology suggest different life styles and adaptations to diverse ecological niches. The pelagic species include many actively swimming fish, but the bottom-living population includes more passive species, some of which have adopted a sit and wait strategy (Merrett 1987). Food resources in the abyss rely less on local productivity, as in the case of volcanic vent communities, and more on the remains of phyto-and zooplankton and larger organisms such as crustaceans, fish, and mammals (whales) (Pinet 2000). Among the 84 demersal species recognized in the North Atlantic basin (Merrett and Haedrich 1997) typical forms include grenadiers (*Coryphaenoides*), eels, and slickheads (*Alepocephalids*).

Knowledge about these fish comes mainly from three sources: (i) trawls with specially designed fishing gear which have produced catches of mainly dead specimens demonstrating the diversity of species and morphological specializations (for review see Merrett and Haedrich 1997), (ii) lander technologies with imaging capabilities that have allowed the observation of fish in their native environment and to study their response to bait (Armstrong et al. 1992; Priede et al. 1994), and (iii) manned and/or unmanned vehicles operating in this environment hostile to human survival (Pinet 2000; Priede and Bagley 2000). Thus, direct observations on the behavior of deep-sea fish are scarce and fragmentary at best. Therefore inferences about the behavior of these animals have been made mostly from dead material.

Collateral to this situation is the fact that very little experimental evidence is available concerning the sensory environment of deep-sea fish. These data are reviewed by Herring (2002) and summarized here. Chemical cues have been used systematically by several groups in the form of bait (dead fish) and a number of different species could be attracted to these stimuli. This would suggest that many species, especially on the bottom of the sea, use these cues to locate food falls and carrion. Olfactory cues have also been implicated in finding female mates for male anglerfish. Incidental observations may further be mentioned, recorded on film or made by observers in submersibles. Tactile orientation behavior using the submandibular barbel as a guiding organ was found in demersal macrourids (I.G. Priede personal communication).

Water flow and pressure resulting from currents or approaching objects (prey/predator; mate) as another mechanosensory stimulus may be assumed to be present and exploited by deep sea fish in similar ways as in other fish. Audition seems to play an important role in communication and orientation (Popper and Fay 1993); anecdotal reports by Marshall indicate that some deep-sea grenadiers do indeed produce sounds audible to humans when brought on board ship (Marshall 1971, 1980), suggesting that they also use it in their native environment. The most intriguing situation concerns vision and optic stimuli. Solar light does not penetrate even the clearest ocean water deeper than 800-1000m with long wavelengths being attenuated earlier, and shorter wavelengths reaching furthest down. The mesopelagic zone is therefore exposed to a very dim blue-green light, to which the visual pigments of most animals are well tuned (Douglas et al. 1998). Bioluminescence supplements or substitutes sunlight as visual cue at mesopelagic and greater depth matching the down-welling sunlight in spectral composition. It is tempting to speculate that the well-developed and even highly specialized eyes in the majority of deep-sea fish (as well as in many crustaceans and cephalopods) have evolved to perceive these stimuli. However, apart from this intuitive conclusion, there is to date no experimental evidence showing physiological responses to light in living retinae (ERGs), nor are there any observations of deep sea fish reacting to light. It must be said, however, that the lights used in these instances were designed to be recorded by cameras or the human visual system, and were therefore possibly of little biological relevance to the fish they were designed to study.

Since a number of previous studies have shown that, in teleosts, the cerebral morphology reflects specializations of their sensory systems (taste: Morita and Finger 1985; Kanwal and Caprio 1987; Finger 1988; electroreception: Heiligenberg 1987) a reverse approach is undertaken here: In a revival of phrenology for fish, the volumes of four sensory brain areas were determined and deductions made about the role of individual senses influencing the behavior in the native environment. For olfaction and vision, correlations between the surface area of the peripheral sensory epithelium and the olfactory lobe or the optic tectum have already been published for shallow water fishes (Kotrschal et al. 1990; Meek and Nieuwenhuys 1997). Exteroception is mediated by the trigeminal (V), lateral line and octaval (VIII) nerves, the axons of which project to the trigeminal/octavolateral area in the dorsal rhombencephalic zone. Afferents of the lateral line organs are contained in the anterior and posterior lateral line nerves and terminate in discrete nuclei within the same area. The contribution of audition is mostly based on saccular and lagenar input to the octaval complex. Knowledge on its contribution to the sensory environment in the deep sea is even more fragmentary than for the other senses; yet its role must not be underestimated. (Marshall 1971, 1980; McCormick 1982; Nieuwenhuys and Pouwels 1983; McCormick and Bradford 1988; Popper and Fay 1993). Gustatory afferents terminate in the intermediodorsal zone of the rhombencephalon; taste buds from the body surface are represented in the facial region, whereas the vagal lobe receives viscero-afferents from the oropharyngeal cavity (Morita and Finger 1985; Kanwal and Caprio 1987; Finger 1988). Together, the facial and vagal lobes will be referred to as gustatory area.

In the present chapter, data from three families of deep-sea fish are considered which have members in both the mesopelagic and the deep demersal habitat. The three families comprise slickheads, eels, and grenadiers, and represent the three most abundant groups in the deep North Atlantic (Merrett and Haedrich 1997). They are part of a larger sample of 67 species of mesopelagic fish (caught in the Eastern North Atlantic and in the Central North Pacific) and from 35 species of deep demersal fish (caught in the Eastern North Atlantic, and in the Porcupine Seabight and Abyssal Plain, southwest of Ireland) of which complete data sets have been previously published (Wagner 2001a, b). The focus on three distinct groups will allow to discuss the impact of environmental constraints on the adaptive differentiation of the sensory brain on the one hand, and the influence of phyletic relationships, on the other.

MEASUREMENTS AND CLASSIFICATIONS

Fish heads were fixed in 4% formalin on board ship (RRS Discovery and FS Sonne). The subsequent dissection exposed the sensory organs, the cranial nerves and the brain on the right half, leaving the left half intact for later reference (Figs. 1-6). In some cases, carbocyanine dyes were applied to the nerves and the projection areas in the brain demonstrated. Back in the laboratory at home, the dorsal and lateral aspects of the brains were recorded with a digital camera. The length, width and depth of every brain were determined using the measuring tool of the Adobe Photoshop 5 program. Values were scaled by referencing them to a mm-scale included in the micrographs. The quantitative analysis basically follows the concepts of Huber et al. (1997) who treated the brain lobes as half-ellipsoids and calculated their volumes from the three cardinal dimensions. In order to discount for size differences and to enable interspecies comparisons the volumes of the four sensory areas were added and the relative proportions determined; in cases where several specimens of a given species were available, average percentages were used. These relative values were very consistent because in all cases, the optic tectum was far greater than the other areas, generally taking up between 50 and 75% of the total volume of the "sensory brain", whilst the other three areas were of more variable sizes. This observation indicates that each sensory system requires neural processing specific to each modality. In the case of the optic tectum, the retinotopic organization associated with topographically arranged feature extraction neurons probably requires more volume than the more random distribution of the odorant-specific glomeruli in the olfactory bulb. Furthermore, whilst the olfactory bulb is only concerned with smell, the optic tectum has been shown to process also inputs other than vision, which may contribute to its expansion in some species (Northcutt 1983; Meek and Nieuwenhuys 1997). The average value of the relative volumes avoids any system-specific bias and identifies the comparative rank of a given species for each sensory system.

This rank is defined with respect to a reference population. One such a population is represented by the environment in which fish have been caught, i.e. the mesopelagic (67 species) and the demersal (35 species) habitat (Wagner 2001a, b). In this case, the relative average of each sensory area within the population serves as baseline for the ranking system (Table 1). Above average cases are represented as + symbols in Table 2, and below average cases as -. Species with only a single sensory area above average are considered as specialists, species with two areas

Table 1 Statistical overview of the distribution of the sensory specialization in the total sample of 67 mesopelagic and 35 demersal species (Wagner 2001a,b) following the average-based analysis of relative volumes with habitat and family as reference sample

	olfactory bulb			optic tectum			V/VIII area			gustatory area		
	mesopel	family	demersal	mesopel	family	demersal	mesopel	family	demersal	mesopel	family	demersal
mean relative volume (%)	2.76		16.48	61.26		57.56	25.32		11.57	10.08		14.38
species above mean (%)	34		37	61		54	41		49	40		34
specialists (%)	1.5		14.2	32.8		20	1.5		0	0		8.6
Alepocephalids (n=11) mean relative volume(%)	5.21	4.01	3.56	60.44	75.6	81.29	19.33	12.04	9.31	15.01	8.32	5.82
Anguilliforms (n=8) mean relative volume(%)	5.3	20.94	47.02	70.25	55.76	31.62	19.74	16.38	10.78	4.76	6.94	10.58
Macrourids (n=9) mean relative volume(%)	2.77	14.93	16.45	63.64	57.68	56.94	21.56	13.32	12.29	12.03	14.05	14.31
Alepocephlid specialists				6	6			1				
Anguilliform specialists		1	2	1	3							
Macrourid specialists		1	1	1	1							

Table 2 Synopsis of relative brain area volumes based on habitat (h) and family (f)

	h	f	olf. bulb h	olf. bulb f	optic tect. h	optic tect. f	V/VIII h	V/VIII f	gust. area h	gust. area f	habitat
Alepocephalidae											
Bajacalifornia megalops	D	G	+	+	+		– –	+	–	+	P
Photostylus pycnopterus	G	G	++	+	–	–	+	+++	+++	+++	P-a
Xenodermichthys copei	D	D	+	+	+	+	– –	–	–	– –	P-s
Alepocephalus productus	S	S	– –	–	+	+	–	–	–	–	D-s/r
Bathytroctes microlepis	S	S	– –	–	+	+	–	–	–	–	D-ls/r
Bellocia koefodi	S	S	– –		+	+	–	–	–	–	D-r/a
Conocara macroptera	S	S	– –		+	+	–	–	–	–	D-ms/ur
Conocara salmonea	S	S	– –		+	+	–	–	–	–	D-a
Conocara sp.	S	S	– –		+	+	–	–	–	–	D-a
Narcetes stomias	D	D	–	+	+	–	+	+	–	–	D-ls/r
Rinoctes nasutus	D	S	– –	–	+	–	+	++	+	–	D-lr/a
Anguilliformes											
Serrivomer beani	D	S	+	–	+	+	–	–	–	–	P
Avocettina infans	D	D	++	–	–	+	+	++	–	–	P-o
Nemichthys curvirostris	S	S	–	– –	+	+	–	–	– –	– –	P-o
Nemichthys spec.	D	S	++	–	+	+	–	–	– –	– –	P-o
Cyema atrum	G	G	++	–	+	+	–	+	+	+	P-b
Histiobranchus bathybius	S	S	+++	++	–	–	–	–	–	–	D-ls/r/a
Iliophis brunneus	D	D	++	+	–	–	+	–	+	+	D-ls/r
Synaphobranchus kaupi	S	D	+++	++	–	–	–	–	+	+	D-us/ur
Macrouridae											
Hymenocephalus metallicus	G	D	+	– –	+	+	–	+	+	–	P-s
Coryphaenoides (Ch.) leptolepis	D	D	–	–	–	–	+	+	+	+	D-ls/r/a
Coryphaenoides mediterraneus	D	D	–	–	–	–	+	+	+	+	D-ms/ur
Coryphaenoides profundicolus	D	D	+	+	–		–	–	+	+	D-a
Caelorinchus labiatus	G	D	+	+	+	+	+	+	–	–	D-s
Coryphaenoides guentheri	G	D	–	–	+	+	+	+	+	+	Dms/ma
Coryphaenoides rupestris	S	S	–	–	–	+	–	–	–	–	D-s
Coryphaenoides (N.) armatus	S	S	+	+	–	–	–	–	–	–	D-r/a
Trachyrincus murrayi	D	D	+	+	+	+	–	–	–	–	D-ms/ls

S specialist: one area above average; D "dominated" species: two areas above average; G generalist- less than relative average; — less than half the rel. mean; — very small, negligible; + more than relative average; ++ more than twice the rel. mean; +++ more than three times the rel. mean habitat information: D demersal; P (meso)pelagic; o ceanic; s continental slope, r continental rise, a abyssal; u upper, m mid, l lower (Merrett et al. 1991, a, b) h habitat as reference; f family as reference

above average are regarded as "<u>dominated</u>" by these two senses, and species with three areas above average are designated as <u>generalists</u> (Wagner 2001a, b). The other frame of reference is provided by the systematic or phyletic position, the families, in the present case the slickheads, eels, and grenadiers. Table 1 shows that there are marked difference between these two approaches of classification; they are also compared in Table 2.

OBSERVATIONS AND DISCUSSION

The **alepocephalids** comprise bottom-living as well as pelagic members; most of them live below 1000m. The present sample consists of three pelagic and eight demersal species. Their diet consists of bottom-living and pelagic invertebrates including crustaceans, tunicates, cteno-phores, polychaetes and echinoderms.

   Bajacalifornia megalops lives between 820 and 1425m (Whitehead et al. 1984). Its head along with views of its brain, and cranial nerves are shown in Fig. 1. Among the sense organs the eyes appear strikingly large and include an aphakic space between lens and iris, which admits light into the bulbus without being refracted by the lens, thus possibly increasing sensitivity. In the brain, the optic tectum clearly dominates all other parts; correspondingly, the optic nerve has the largest diameter among the cranial nerves. The percentages of volumes of the four main sensory areas are represented as bar diagram (inset Fig. 1B); as references, the habitat based averages are indicated as solid lines and the family based averages given as broken lines (see Table 1). The relative volume of the optic tectum amounts to about 75% corresponding closely to the family based average. Interestingly, the relative volume of the stalked olfactory bulb exceeds both the habitat and the family based averages. *Conocara macroptera* (Fig. 2) belongs to the demersal community (Merrett and Haedrich 1997). At a first glance, the head, including the sense organs, and the brain with the cranial nerves closely resemble those of *B. megalops*. In quantitative terms, the dominance of the optic system is even greater in *C. macroptera* than in *B. megalops* since the individual volumes exceeds both the habitat and the family based averages (Fig. 2B inset; Table 1). The preponderance of the visual system in this species has been shown previously in a comparative study of the olfactory and the visual system (Collin et al. 2000). Like a number of other alepocephalids, the eye of *C. macroptera* not only contains an aphakic gap, but also a foveate retina in which the number of rod banks is markedly increased (Wagner et al. 1998; Collin and Partridge 1996; Locket 1985).

   The group of eleven slickheads is more homogeneous in its sensory orientation than either of the two others. With the exception of *Photostylus pycnopterus* they are characterized by an above-average optic tectum in the habitat context. The family based average of this area is far higher than the habitat based value; yet seven species have optic tecta exceeding even this threshold. The dominant role of vision in slickheads is independent of their habitat because it is found in the demersal species as well as in two of the three pelagic specimens. It is not only manifest in the size of the eyes, and the aphakic space but also in numerous specializations of retinal morphology. Most notably, they possess a convexiclivate fovea (Munk 1968, Locket 1971, 1985; Collin and Partridge 1996, Wagner et al. 1998, Collin et al. 2000) including special arrangements of radial glia (increased refraction), higher numbers of rod banks (increased
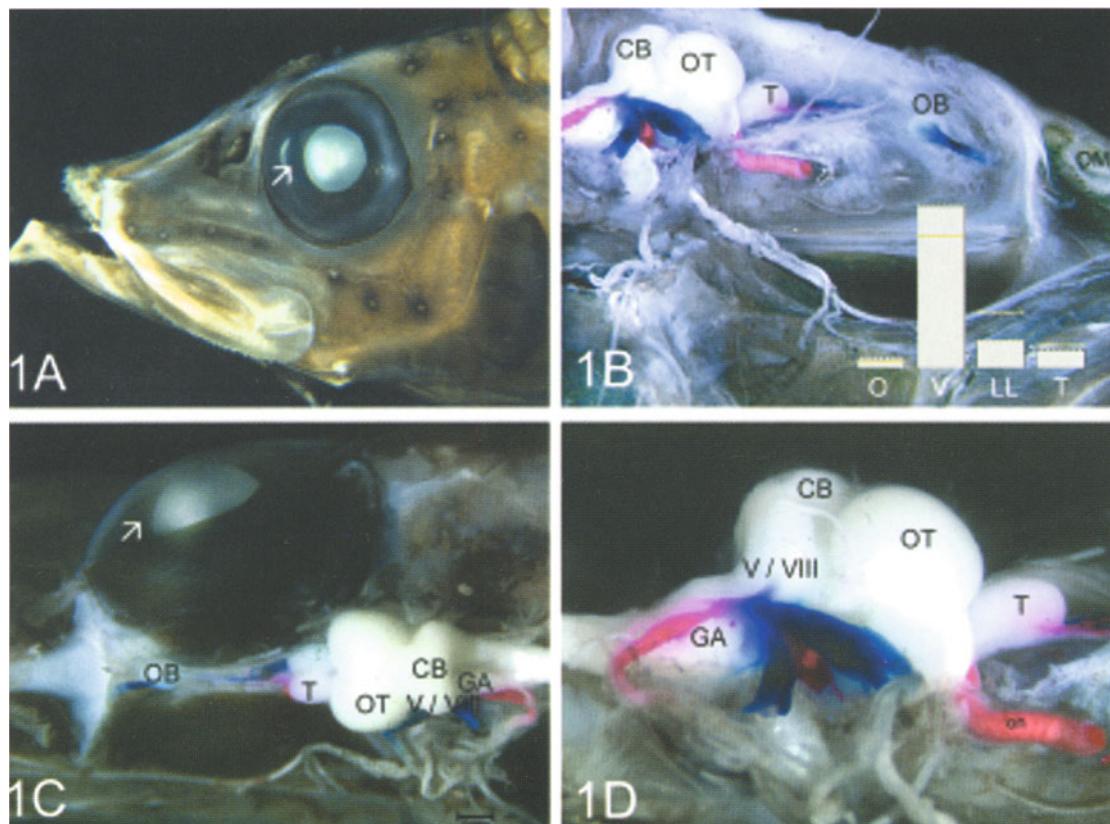
**Fig. 1** *Bajacalifornia megalops* B: Dissection of brain and cranial nerves, lateral aspect; C: dorsal aspect; D: lateral view of brain; some cranial nerves are stained with carbocyanine dyes. Note the stalked olfactory bulb (OB). CB cerebellum; GA gustatory area; OM olfactory mucosa; OT optic tectum; on optic nerve ; T telencephalon; V / VIII trigeminal/octavolateral area. The arrows point to the aphakic gap between iris and lens; the empty arrow (1B) indicates the eyestalk. The bar diagram represents the relative volumes of the olfactory bulb (O), the optic tectum (V), the octavolateral area (LL), and the gustatory area (T) in percent; lines indicate habitat based averages; broken lines indicate family based averages.

sensitivity), and a high density of ganglion cells in the parafoveal region (increased spatial resolution). While *Bajaclifornia*, *Photostylus* and *Xenodemichthys* are bioluminescent themselves, the other slickheads are not. The highly differentiated visual system may be used for predation of often bioluminescent crustaceans, jellyfish and salps (Herring 1987; Mauchline and Gordon 1985; Crabtree and Sulak 1986), and it may play an additional role in intraspecific communication in the first three species. An interesting distinction between the pelagic and demersal species can be seen in the senses associated with vision: In two of the demersal species the octavolateral system plays an important additional role, whereas olfaction is consistently above average in the (meso)pelagic species. *P. pycnopterus* is the only non-visual slickhead in our sample. It has a dominant gustatory system, associated with above average olfaction and also
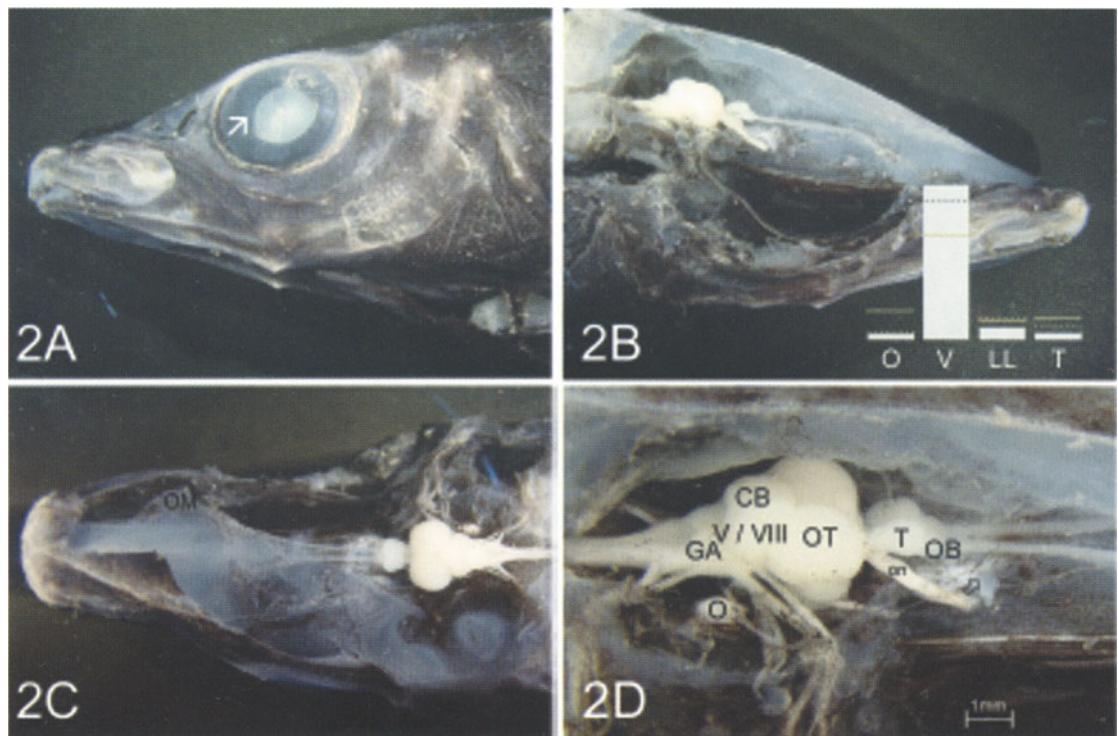
Fig. 2 *Conocara macroptera* B: Dissection of brain and cranial nerves, lateral aspect; C: dorsal aspect; D: dorso-lateral view of brain. Note the stalked olfactory bulb (OB). CB cerebellum; GA gustatory area; OB olfactory bulb; OM olfactory mucosa; OT optic tectum; on optic nerve; O otolith; T telencephalon; V / VIII trigeminal/octavolateral area. The arrow points to the aphakic gap between iris and lens; the empty arrow (2D) indicates the eyestalk. The bar diagram represents the relative volumes of the olfactory bulb (O), the optic tectum (V), the octavolateral area (LL), and the gustatory area (T) in percent; lines indicate habitat based averages; broken lines indicate family based averages.

octavolateral system. Unfortunately, nothing is known about its diet or its detailed habitat, except that it is found deeper than the other two pelagic species (i.e. below 1000m; Whitehead et al. 1984).

The **anguilliform eels** form a heterogeneous group of mostly pelagic fish some of which have been found over a wide range of depths. Among the eight species of the present sample five live predominantly in the water column from epipelagic depths to about 2000m; they feed mainly on crustaceans (Whitehead et al. 1984). The remaining three species belong to the synaphobranchid eels, one of the dominant groups of the Northern Atlantic demersal fish fauna (Merrett and Haedrich 1997); they live on the abyssal slope or plain and are active piscivorous scavengers.

Serrivomer beani (Fig. 3) has an elongated, pointed head, with a brain situated several cm behind the eyes, leading to unusually long olfactory and optic nerves (Fig. 3B). Like the slickheads, the eyes have aphakic spaces, but in this case both rostrally and caudally. Among the
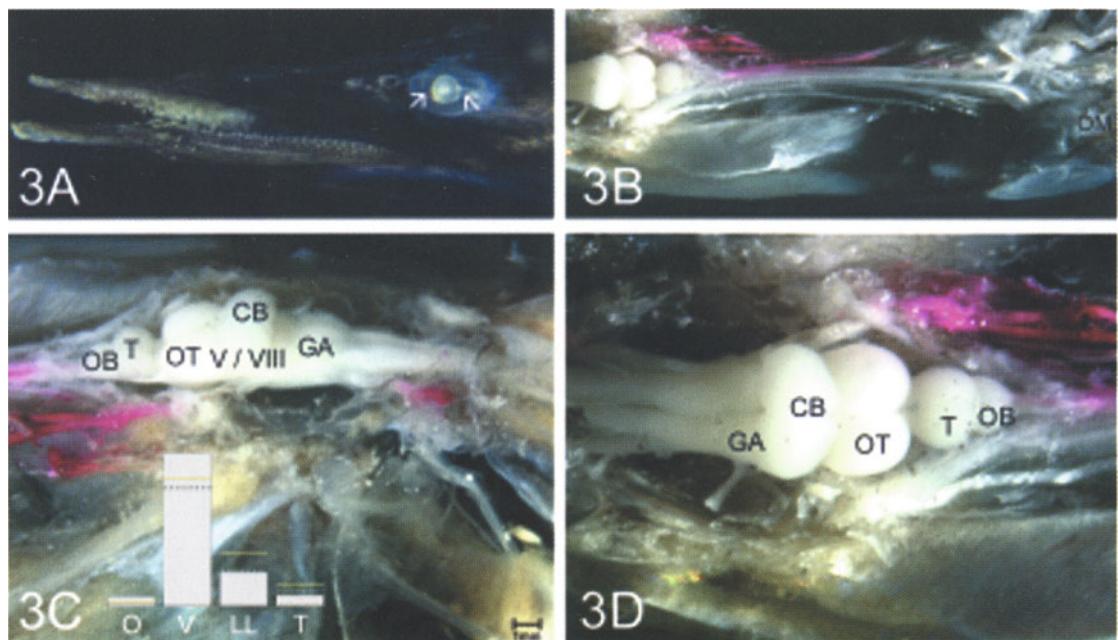
Fig. 3 *Serrivomer beani* B: B: Dissection of brain and cranial nerves, dorsal aspect; C: dorsolateral aspect; D: dorsal view of brain; some cranial nerves stained with carbocyanine stains. Note the long distance between the brain and the eyes and olfactory rosette. CB cerebellum; GA gustatory area; OB olfactory bulb; OM olfactory mucosa; OT optic tectum; T telencephalon; V / VIII trigeminal/octavolateral area. The arrows point to the aphakic gaps between iris and lens. The bar diagram represents the relative volumes of the olfactory bulb (O), the optic tectum (V), the octavolateral area (LL), and the gustatory area (T) in percent; lines indicate habitat based averages; broken lines indicate family based averages.

sensory brain areas, the optic tectum occupies the largest volume clearly exceeding habitat and family based averages and, with about 73.8% almost reaching the levels of alepocephalids. In addition the octavolateral complex about equals the family based mean (Fig. 3C, inset; Table 1). The demersal *Ilyophis brunnei* (Fig. 4) differs in many respects from *S. beani*. The external view shows a long distance between the inlet and outlet openings of the nose (Fig. 4A); this corresponds to a very conspicuous differentiation of the olfactory mucosa, which consists of stacks of closely spaced lamellae (Fig. 4B). Correspondingly, the olfactory bulbs are large and arranged so one lies on top of the other (Fig. 4C). In quantitative terms, the relative olfactory bulb volume is only 6% lower than that of the optic tectum (35% vs. 41%) and is markedly higher than both types of averages (Fig. 4D, inset; Table1). The octavolateral and the gustatory areas are small and of similar size.

The differences between *S. beani* and *I. brunnei* also hold in a more general way for the two groups of eel. Like *S. beani*, *Avocettina infans*, the two *Nemichtys* species and *Cyema atrum* are all pelagic and, except for *C. atrum*, found between 150 and 2000m. All have well developed visual systems; *A. curvirostris* is even a visual specialist. The other species appear to use vision in combination with olfaction, the octavolateral complex, and, in the case of *C. atrum*, with
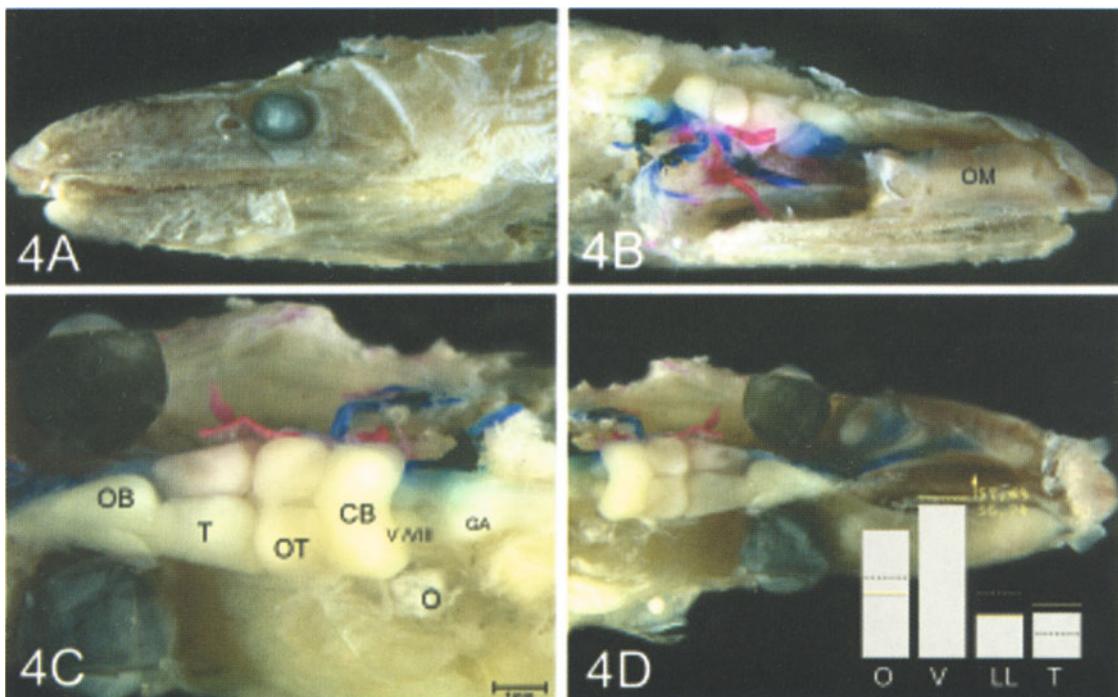
Fig. 4   *Ilyophis brunneus* B: Dissection of brain and cranial nerves, lateral aspect; C: dorsal aspect; D: dorsal view of brain; some cranial nerves stained with carbocyanine stains. Note the voluminous olfactory bulb(OB), and mucosa (OM), composed of extensive stacks of olfactory lamellae. CB cerebellum; GA gustatory area; O otolith; OT optic tectum; T telencephalon; V / VIII trigeminal/octavolateral area. The bar diagram represents the relative volumes of the olfactory bulb (O), the optic tectum (V), the octavolateral area (LL), and the gustatory area (T) in percent; lines indicate habitat based averages; broken lines indicate family based averages.

gustation characterizing them as "dominated" or generalists (*C. atrum*) (Table 2). Since all of these mostly mesopelagic eels have been shown to feed predominantly on crustaceans (Whitehead et al. 1984) one may conclude that predation will be mostly visually guided, relying either on residual sunlight or bioluminescence. By contrast, the demersal synapho branchid eels are among the most prominent olfactory specialists with olfactory bulbs exceeding two or three times the average in *Histiobranchus bathybius* and *Synaphobranchus kaupi* (Table 2). They are large mobile piscivorous scavengers that appear quickly and in high abundance at food falls (Jannasch 1978; Merrett and Domanski 1985). The present observations convincingly demonstrate that these deep-sea eels are above all "swimming noses" and explain the effectiveness with which they locate their prey.

The **macrourid grenadiers** comprise more than 300 species and are the most speciose deep-demersal family; most of them live in the boundary layer above the bottom and are characterized as benthopelagic. In our sample of nine species, *Hymenocephalus metallicus* and *Coryphaenoides mediterraneus* live in areas of the upper slope, between 800 and 2000m. Species richness is greatest in low latitudes on the slope. On the other hand, *C. (N.) armatus*

and C. *(Ch.) profundiculus* occupy the deepest end of the habitat, from 2,000m downwards to the abyssal plain. Many slope dwelling species, among them *H. metallicus*, are bioluminescent ventrally (Herring 1987). Feeding strategies vary greatly including active scavengers, carrion eaters and euryphagous species.

The meso-benthopelagic *H. metallicus* has large eyes including an aphakic space and an inconspicuous nose (Fig. 5A). This corresponds to a larger than family average optic tectum and a small olfactory bulb (stalked) (Fig. 5C, Table 1). The octavolateral and gustatory areas are intermediate between the two types of average volumes. Compared to the sensory areas, the cerebellum dominates all other parts of the brain (Figs. 5 B, D). In *C. rupestris*, the eyes are also large and include a narrow aphakic space (Fig. 6A). The relative volume of the optic tectum is even larger by 5% than in *H. metallicus* (Fig. 6C, Table 1); the size of the olfactory bulb is also
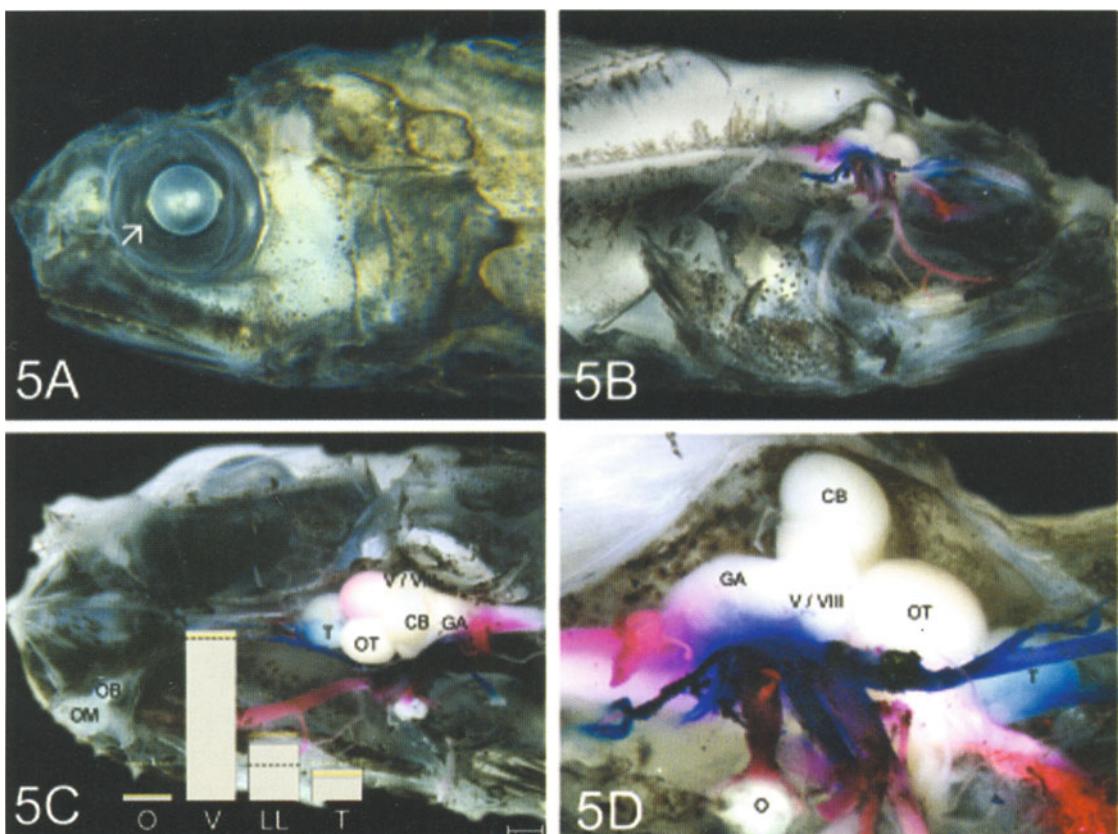
Fig. 5 *Hymenocephalus metallicus* B: Dissection of the brain and cranial nerves, lateral aspect; C: dorsal aspect; D: lateral view of brain; some cranial nerves stained with carbocyanine stains. Note the long olfactory tract and the stalked olfactory bulb (OB). CB cerebellum; GA gustatory area; OM olfactory mucosa; OT optic tectum; T telencephalon; V / VIII trigeminal/octavolateral area. The arrow points to the aphakic gap between iris and lens. The bar diagram represents the relative volumes of the olfactory bulb (O), the optic tectum (V), the octavolateral area (LL), and the gustatory area (T) in percent; lines indicate habitat based averages; broken lines indicate family based averages.
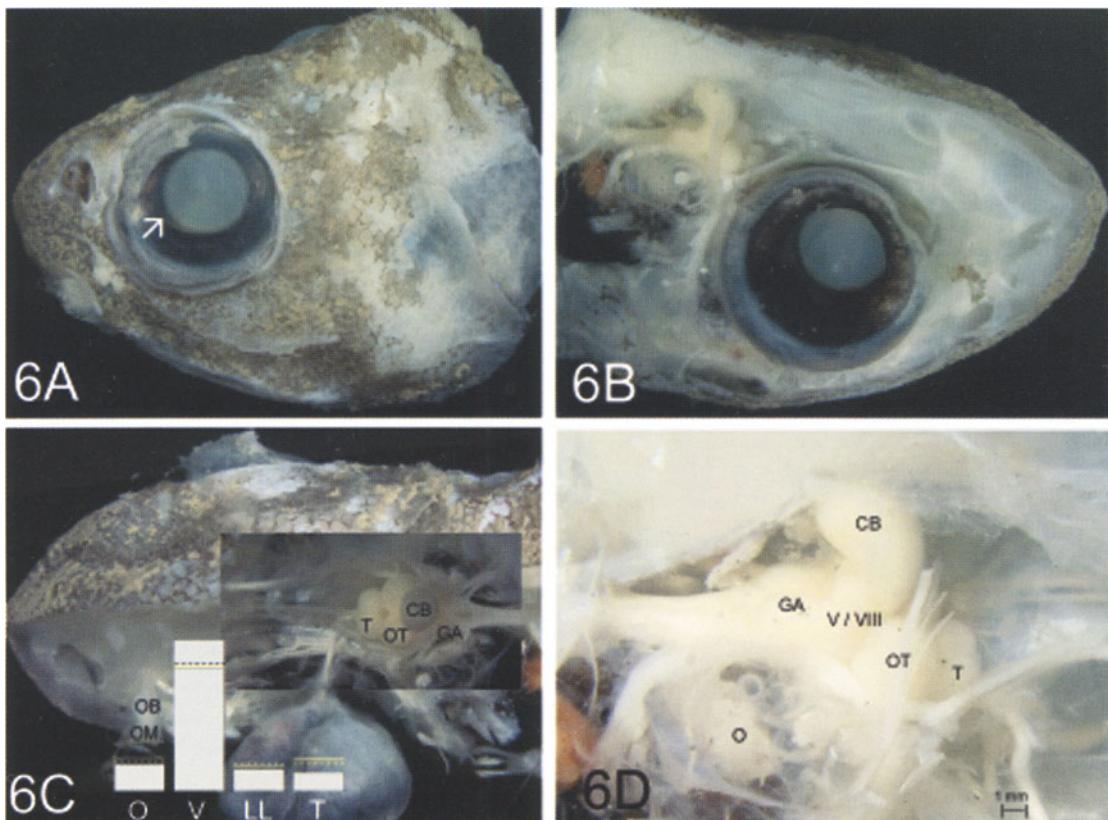
Fig. 6 *Coryphaenoides rupestris* B: Dissection of the brain and cranial nerves, lateral aspect; C: dorsal aspect; D: dorsolateral view of brain. Note the long olfactory tract and the stalked olfactory bulb (OB) . CB cerebellum; GA gustatory area; OM olfactory mucosa; OT optic tectum; T telencephalon; V / VIII trigeminal/octavolateral area. The arrow points to the aphakic gap between iris and lens. The bar diagram represents the relative volumes of the olfactory bulb (O), the optic tectum (V), the octavolateral area (LL), and the gustatory area (T) in percent; lines indicate habitat based averages; broken lines indicate family based averages.

larger and even exceeds the octavolateral and gustatory areas. Similar to *H. metallicus*, and most other grenadiers, the cerebellum is the largest part of the brain (Wagner 2001b).

Considering, in addition, the remaining seven grenadier species (Tables 1, 2) it emerges that the sensory differentiations are more heterogeneous in this family than in the other two. Although there is one specialist for olfaction (*C. [N.] armatus*) and vision (*C. rupestris*) according to both kinds of averages, the octavolateral system and gustation also play major (above average) roles (together in *C. leptolepis, mediterraneus, guentheri;* combined with above average olfaction and vision in *Caelorinchus labiatus*), resulting in the classification of seven "dominated" species in the family context, and four "dominated" plus three generalists in the context of habitat-based averages (Table 2). This corresponds to highly diverse behaviors including feeding strategies. On the one hand, there are the macronekton foragers such as the more

pelagic *Coryphaenoides rupestris* from the continental slope and the more benthic *C. guentheri* from the continental rise that feed on small prey, that are often schooling off the bottom (Gartner et al. 1997). They are never attracted to bait placed on the sea floor (Priede et al. 1990, 1994). This corresponds well to the below-average olfactory bulbs in these two species; instead, they have an above-average optic tectum suggesting that visual cues play a more prominent role in locating prey, possibly linked to bioluminescent signals emitted by the targets. *Trachyrincus murrayi* prefers active, pelagic quarry (Priede et al. 1999); the present observations indicate that this species relies on a combination of visual and olfactory stimuli for feeding. The highly abundant *C. (N.) armatus* from the continental rise and the abyssal plain is a truly euryphagous species feeding on live and dead, pelagic and bottom-living animals (Sedberry and Musick 1978). It rapidly locates carrion by virtue of its olfactory system and is one of the first to appear when baited cameras are deployed (Wilson and Smith 1984; Armstrong et al. 1992). In the present context of comparative sensory brain areas, *C. armatus* is the only olfactory special-ist among the grenadier fishes. Preliminary counts of axons in the olfactory tract and optic tract in this species show 5.9 times more olfactory than optic fibers thus confirming the leading role of the olfactory system (S.P. Collin and H.-J. Wagner in preparation).

CONCLUSIONS

Senses and the Habitat

There are significant differences between the mean volumes of the sensory areas in the two habitats. The mesopelagic population has reduced olfactory bulbs, a larger optic tectum and an octavolateralis area more than twice as large as in the demersal population. In the latter group, by contrast the chemical senses, above all the olfactory bulb seem to play a much larger role (Table 1). This would indicate that olfaction is considerably more important on or near the bottom than in the open water, where vision, the lateral line system including, possibly, audition have a higher cerebral representation. This conclusion is further supported by the number of (habitat based) specialists: among the demersal species, there are 14% olfactory and about 9% gustatory specialists vs. 1.5% and none in the mesopelagic group; on the other hand, about one third of the mesopelagic species are visual specialists, as opposed to one fifth in the demersal group (Table 1). If one takes into account the distribution of preferred senses in the "domi-nated" species and the generalists from both habitats the above trend can also be found (Wagner 2001a, b). The dominant role of vision in the mesopelagic environment is linked to the fact that residual solar light is still available above 1000m and that bioluminescence is common in the mesopelagic fauna. In the present sample, all of the mesopelagic slickheads as well as the grenadier are bioluminescent; whilst the eels are not. However looking at the entire population, a simple correlation between bioluminescence and a highly developed optic tectum cannot be detected (Wagner 2001a). On the one hand, there are bioluminescent species like the hatchetfish many of which have a large optic tectum; other bioluminescent species, how-ever, like the anglerfish and most of the lanternfishes (myctophids) have sub-average or even reduced optic tecta. On the other hand, there are visual specialists that are not bioluminescent

themselves, like many eels, which are supposed to hunt for luminous prey. Quantitatively, among 43 bioluminescent mesopelagic species, there are 25 with above average visual tecta, including twelve visual specialists, eleven visually "dominated" species, and two generalists with a visual component. In the remaining 43% (18), the relative optic tectal volume is below average; from the 28 non-luminous species, eight are visual specialists, five are "dominated" and there is a single generalist with a visual component, whilst eight have a smaller than average optic tectum.

The sensory environment of the abyss is markedly different from the open waters of the deep sea. Although bioluminescence is also present near or on the bottom of the deep sea (Herring 1987) it seems to be less abundant; instead, currents and possibly sounds appear to play a more prominent role in the demersal habitat. The importance of audition in the abyss has been discussed for macrourids, morids and ophidiids (Marshall 1971; Merrett and Haedrich 1997; Montgomery and Pankhurst 1997). Chemical cues seem to be more important near the bottom than in the mesopelagic environment. This may be correlated to the fact that carcasses of animals from the upper layers end up here and play a major role as food source. A highly differentiated olfactory system is instrumental in the localization of carrion, and has been recorded in a number of species of the present sample by baited cameras and lander systems (Priede et al. 1990, 1994; Priede and Bagley 2000).

Senses and Family

Apart from environmental factors which play an essential role in shaping brain structures by way of adaptive and exaptive processes (Northcutt 1988), phylogeny expressed as the systematic position must also be taken into account. Previous attempts to integrate these two evolutionary principles in studies of teleost brains have been undertaken by Albert et al. (1998), and Eastman and Lannoo (1998).

Among the three families of teleosts considered in this chapter the anguilliform eels belong to the non-euteleost elopomorphs, the alepocephalids are part of the euteleost salmoniforms, whilst the grenadiers are potentially most advanced and grouped among the neoteleost gadiforms (Nelson 1984). Considerations about primitive and derived features in deep sea fish have to be treated with caution, however, in view of the complex processes involved in the phylogeny of this ichthyofauna. Andriyashev's (1953) hypothesis of an ancient population of primitive teleosts supplemented by secondary forms from derived phylogenetic groups that moved into the deep water later has been complemented by the "deep allopatry hypothesis" (White 1987) which makes recurrent periods of anoxic events responsible for isolation and speciation in a dynamic environment. Indeed, the interchange between especially the pelagic and the demersal ichthyofauna has been found to be remarkably low (Merrett 1994).

The sensory orientation of the salmoniform slickheads is very uniform because in the habitat based reference system they have an above average optic tectum in common. This predominant visual orientation is somewhat mitigated in the family based context because the mean volume of the slickhead optic tectum amounts to 75%. As noted above the visual system of alepocephalids has long been known for its special features (eyestalks, aphakic spaces, retinae

with grouped or multibank photoreceptors in addition to specialized areas such as convexiclivate foveae [Munk 1968; Locket 1971, 1985; Collin and Partridge 1996; Wagner et al. 1998; Collin et al. 2000]) which have been interpreted as adaptations to complex and highly evolved predatory behaviors. The dominant role of vision is retained irrespective of the pelagic or demersal origin and it appears difficult to suggest a causal explanation for the fact that the mesopelagic species of the present sample have recruited olfaction as an additional above average modality, whereas the octavolateral system has additional significance in two of the demersal species. The exceptional position of *P. pycnopterus* which seems to prefer all other senses to vision cannot be explained by any additional data, yet.

In the anguilliforms, the constraints exerted by the phyletic adherence appear less strong than those of the environment. All of the mesopelagic species have an above average optic tectum (at least by family standards) suggesting that vision plays a major role in their behavior. By contrast none of the three demersal eels seems to rely on vision; they are all characterized by olfactory bulbs that are twice or even three times as big as the average. In the habitat based classification two of them are olfactory specialists and additional senses are used only to a lesser degree. Many of the mesopelagic eels, on the other hand, combine vision with additional senses; thus most of them are dominated by two modalities or are generalists. The differentiation of the sensory systems thus shows a much broader spectrum than in the slickheads; it seems to have evolved as an adaptation to the actual habitat of a given species.

Similar considerations as in the eels would also hold for the grenadiers. Unfortunately, there is only a single species from the upper water layers contained in the present collection. It is all the more interesting to note the great diversity of sensory orientation in the population of demersal macrourids. Although there are some differences between the habitat and the family based classification it is obvious that on both scales the use of the various senses is distributed about evenly, both singly and in combination, resulting in similar numbers of specialists, dominated species and generalists. The distinct lack of dominance of any single sensory modality would argue in favor of a common trait of the grenadier family which enables them to adapt to the diverse ecological niches afforded by the demersal habitats and to develop individual, species specific strategies of sensory specialization and resulting behaviors to interact with theses environments.

In summary, the study of sensory brain areas leads to deductions about the kinds of sensory modalities used preferentially in an environment such as the demersal and the mesopelagic habitat, and would indicate that both regions present fish with rich and diverse sensory environments. While vision emerges as the dominant sense of the mesopelagic realm, olfaction seems more important on or near the bottom of the sea. However other sensory modalities supplement these senses in species specific patterns. Considerations of the phyletic relationships indicate different degrees of pervasiveness. Whilst in slickheads the dominance of vision appears to be a family related trait, a similar relationship is not found in either eels or grenadiers. By contrast, the common trait in these two families seems to be the greater adaptability to the environment.

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Neural Mechanisms of Hearing in Fishes

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ABSTRACT

Our understanding of the sense of hearing in fishes has been greatly enhanced in recent years. This chapter selectively reviews important findings of neural mechanisms of hearing in fishes including both hearing specialists and generalists. It first introduces basic knowledge of physical properties of underwater sounds and structure of fish's auditory organs, and then describes sound detection pathways in different fish species. Major auditory nuclei and their connections that are involved in central auditory processing are also included. Research on fish hearing has previously focused on neural representation of simple sounds, with the majority of studies done on a few species, particularly the goldfish (*Carassius auratus*). This review summarizes transformations of temporal response patterns and changes of frequency selectivity at several levels of the goldfish's auditory pathway. Recent studies of auditory research in fishes have aimed at the understanding of neural coding of biologically relevant sounds such as those produced by fishes themselves. Males of the mormyrid *Pollimyrus adspersus* and midshipman *Porichthys notatus*, two of many vocalizing fishes, produce species-specific sounds for acoustic communication during courtship behavior. Although we do not fully understand how the communication sounds are processed in the brains of these species, some experimental evidence has begun to uncover mechanisms of neural coding of acoustic signals that resemble their courtship sounds. At the end of the chapter, hypotheses and experimental data regarding how fish localize a sound source are reviewed. Sensory hair cells in fish's hearing organs are spatially oriented in three dimensional space, and these hair cells are most sensitive to detecting a sound traveling along the directions of their morphological polarizations. These have led us to believe that each sensory hair cell in the fish's ear is a directional sensor. Experimental results have revealed that response directionality of auditory afferents correlates significantly with morphological polarity of the hair cells innervated by them, indicating that the response directionality of hair cells is faithfully relayed to the brain via auditory afferents. However, it is still not clear how peripherally encoded directional information, the axis at which a sound propagates, is further processed in the brain to extract the actual direction of the sound source.

Key words: Hearing, Frequency selectivity, Otholotic organ, Sound localization, Temporal response

INTRODUCTION

Underwater Acoustics

It is essential to understand underwater acoustics for the study of hearing in fish. A sound wave, either in air or water, has both pressure and particle motion components that are associated with each other. Sound intensity can be measured in either pressure or particle motion, which can be expressed in displacement, velocity, or acceleration. Particle motion, a vectorial quantity, carries directional information of the sound source as well as sound intensity, while sound pressure, a scalar quantity, only provides sound intensity without any directional information. Both particle motion and pressure diminish over distance away from the sound source. In the range near the sound source (called the acoustic near field), particle motion decreases much faster ($1/\text{distance}^2$ for a monopole source such as an underwater loudspeaker, $1/\text{distance}^3$ for a dipole source such as the fish tail movement) than sound pressure ($1/\text{distance}$ for the monopole source, $1/\text{distance}^2$ for the dipole source). Beyond the near field (called the acoustic far field), both particle motion and pressure decrease at a rate of $1/\text{distance}$. The boundary between the near and far fields for a dipole sound source is generally defined as λ/π, about one third of the wavelength (λ), where $\lambda = c/f$, c is sound speed, and f is sound frequency (Siler 1968). The size of the near field is inversely proportional to sound frequency so that a lower-frequency sound source has a larger near field than a high-frequency one, and vice verse. For example, the near field of a 100-Hz dipole source in water extends 5 m from the sound source. Detailed information of underwater acoustics was described by Kalmijn (1988) and Rogers and Cox (1988).

Hearing Organs

Fishes have the lateral line and auditory systems that are involved in sound detection and localization. The lateral line is sensitive to the differential movement between the fish's body and surrounding water that occurs in the very near field (within one or several body lengths), rather than back-and-forth particle motion that is associated with a pressure wave (Kalmijn 1988). Previous studies have shown that the cod (*Gadus morhua*) requires two intact ears, but not the lateral line system, to make directional discriminations (Schuijf 1975, Schuijf and Siemelink 1974). In general, it appears that the lateral line system of fish may be involved in sound detection and localization in the very near field, while the auditory system is primarily responsible for detecting and localizing sound sources in both the near and far fields.

Unlike terrestrial vertebrates having pairs of external, middle, and inner ears, fish only have a pair of inner ears. Each ear of jawed fishes is composed of three semicircular canals, three ampullae, and three otolithic organs such as the lagena, saccule, and utricle (see Fig. 1A and B). Otolithic organs are considered hearing organs though they may also contribute to the vestibular function. Since fishes are evolutionarily old vertebrates, their auditory and vestibular functions are not clearly separated. In addition, the macula neglecta, a sensory epithelium, in the elasmobranch's ear also plays an important role in hearing (Fay et al. 1974, Corwin 1977).
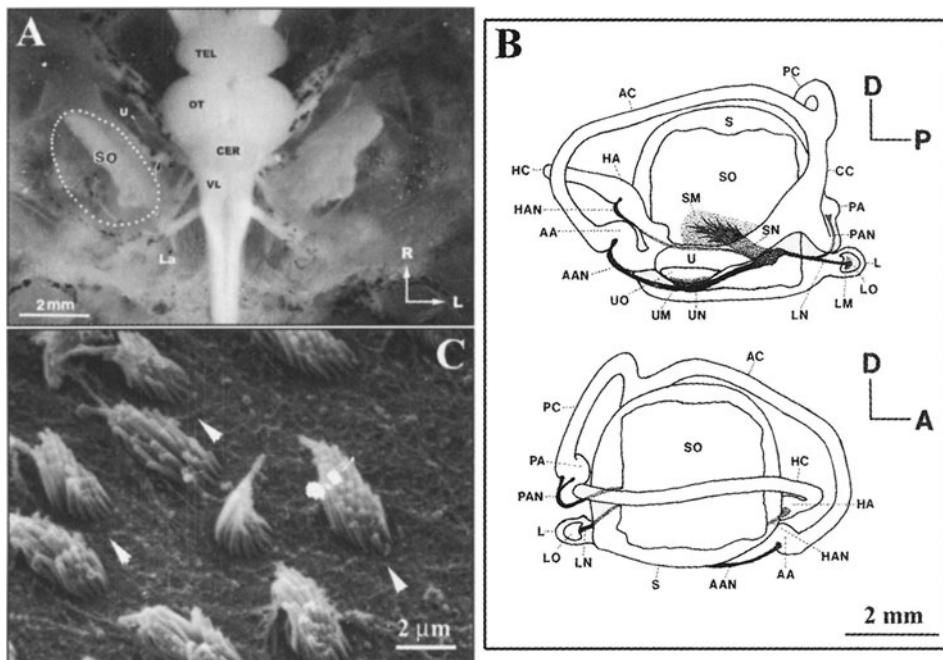
Fig. 1 (A) Photo of the sleeper goby's brain and ears from the top view. *CER* cerebellum, *La* lagena, *OT* optic tectum, *SO* saccular otolith, *U* utricle, *VL* vagal lobe, and *TEL* telencephalon. The saccule is highlighted by *dots*. The saccular nerve bundle is indicated by the *arrow*. Note that the semicircular canals are out of focus. *L* lateral, *R* rostral. (B) Ear of the sleeper goby from the medial view (*top*) and the lateral view (*bottom*). *L* lagena, *S* saccule, *U* utricle, *AA* anterior ampulla, *AC* anterior semicircular canal, *CC* common canal, *HA* horizontal ampulla, *HC* horizontal canal, *LM* lagenar macula, *LN* lagenar nerve, *LO* lagenar otolith, *PA* posterior ampulla, *PC* posterior canal, *SM* saccular macula, *SN* saccular nerve, *SO* saccular otolith, *UM* utricular macula, *UN* utricular nerve, *UO* utricular otolith, *AAN* anterior ampullar nerve, *HAN* horizontal ampullar nerve, *PAN* posterior ampullar nerve. *A* anterior, *D* dorsal, and *P* posterior. (C) Scanning electron microscopic photo of ciliary bundles of sensory hair cells in a saccular macula. Polarizations of three hair cells are indicated by *arrowheads*. (A from Lu and Popper 2001, with permission, © 2000 Springer-Verlag New York Inc.; B and C from Lu and Popper 1998, with permission, © 1998 Elsevier Science B. V.)

An otolithic organ is a fluid-filled sac with a thickened sensory epithelium that connects to a calcareous otolith via an otolithic membrane. Each macula contains many sensory hair cells, which are innervated by a branch of the eighth nerve (the saccular, lagenar, or utricular nerve) (Fig. 1B). A ciliary bundle at the apical end of each hair cell consists of a single eccentrically-placed kinocilium and a cluster of stereocilia that often get shorter the further they are from the kinocilium (Flock 1964). The morphological polarization of a hair cell runs from the stereocilia through the kinocilium (see Fig. 1C). When a sound wave reaches the fish's ear, it generates relative motions between the sensory epithelium and otolith because their significant density difference results in different inertia. Sensory transduction occurs when the relative motions deflect ciliary bundles of hair cells (Hudspeth and Corey 1977). This transduction mechanism appears to be shared by all vertebrates.

Pathways of Sound Detection

Fishes are generally categorized into two groups based upon their hearing capacities (Fay 1988 for a review). Hearing specialists, including otophysans (the goldfish, *Carassius auratus*), mormyrids, and clupeids, have specialized auditory accessories (the Weberian ossicles connecting the swim bladder to their inner ears, or gas-filled structure acoustically coupled to the ears) to enhance hearing sensitivity and broaden frequency response range, while hearing generalists such as the toadfish (*Opsanus tau*) and sleeper goby (*Dormitator latifrons*) have relatively low hearing sensitivity to sound pressure and a narrow frequency response range due to the lack of the specialized auditory accessories and a relatively far distance between the swim bladder and inner ears.

A sound wave likely stimulates the fish's ear via two distinct pathways (Schuijf and Buwalda 1980, Popper et al. 1987 for reviews). The particle motion component may directly reach the ear of a fish through its skull (called the "direct" pathway) because the density of body tissue is similar to that of surrounding water. This direct stimulation appears to apply to all fishes including hearing specialists and generalists. For hearing specialists, the pressure component of the sound wave may indirectly stimulate the inner ear via the swim bladder or gas chambers (called the "indirect" pathway). Thus, hearing specialists are sensitive not only to the particle motion input through the direct pathway, but also the pressure input of the sound via the indirect pathway. In contrast, most hearing generalists, particularly those having no swim bladder or their swim bladders relatively far away from their ears, are thought to be primarily sensitive to the particle motion.

Pressure and Particle Motion Measurements

For auditory research in fishes, a loudspeaker under water or in air has been traditionally used to provide acoustic stimuli (Furukawa and Ishii 1967, Fay 1978a, b, Fay and Ream 1986, Coombs and Fay 1987, Crawford 1993, 1997, Lu and Fay 1993, 1995, 1996, Bodnar and Bass 1997, Kozloski and Crawford 1998, 2000). Although a sound generated by the loudspeaker contains both pressure and particle motion components, sound pressure is usually calibrated and monitored using a hydrophone to interpret results. This implementation is a convenient way to produce and measure sounds and suitable for experiments on hearing specialists that are highly sensitive to sound pressure. However, it is not clear if the pressure measurement is also appropriate for hearing generalists whose ears are considered particle motion detectors rather than sound pressure receivers.

A few studies on fish hearing have been conducted by measuring particle motion (Sand 1974, Schuijf 1975, Fay 1984, Lu et al 1996, Schellart et al. 1995). Since particle motion carries directional information about a sound source, research aims have been directed at understanding how the fish auditory system encodes the particle motion in order to determine the direction of the sound source. An experimental device, the shaker table, was developed by Sand (1974) to provide linear accelerations that mimic underwater particle motion in two dimensional space. The apparatus was later re-designed by Fay (1984) to generate directional stimuli in three

dimensional space. The shaker apparatus has since been used to study directional hearing in several teleost fishes (see DIRECTIONAL HEARING on page 160).

Auditory Pathways

Central auditory pathways have been studied in many fish species using neuronal tracing techniques (Northcutt 1980, McCormick 1981, Finger and Tong 1984, Echteler 1984, Striedter 1991, McCormick and Braford 1993, 1994, Highstein et al. 1992, Edds-Walton 1998, O'Marra and McCormick 1999, McComick 1999 for a review). In teleosts, there are seven first-order octavolateral nuclei in the medulla: anterior octaval, descending octaval, magnocellular, posterior octaval, tangential, medial, and caudal nuclei. The first five nuclei primarily receive inputs from the eighth nerve whereas the last two receive major inputs from the lateral line mechanoreceptors. Although all three otolithic organs project to the descending octaval nucleus (DON) and their projection sites overlap, the inputs to the dorsomedial zone of the DON appear to be generally organized in medial to lateral columns. In many fishes except clupeids, saccular, lagenar, and utricular projections terminate in medial, intermediate, and lateral regions of the dorsomedial zone of the DON (see McCormick 1999 for a review).

Neurons in the dorsomedial zone of the DON in all fish species and the anterior octaval nucleus (AON) in some species project to nucleus centralis (NC), the dorsomedial zone of the torus semicircularis in the midbrain, which is homologous to the mammalian inferior colliculus. In both hearing specialists and generalists, neurons in the dorsomedial part of the DON project to NC either directly or via the secondary octaval population (McCormick and Hernandez 1996, Kozloski and Crawford 1998, Bass et al 2000), which is analogous to the mammalian superior olive.

NC provides a descending input to the DON directly, or in otophysans such as the goldfish, via the medial pretoral nucleus. NC projects to various diencephalic targets (Striedter 1991, Braford et al. 1993, Kozloski and Crawford 1998, Bass et al. 2000), including the central posterior nucleus (CP) of the dorsal thalamus, a portion of the preglomerular complex, and, in some species, the anterior tuberal nucleus of the hypothalamus. The auditory part of the preglomerular nucleus projects to the area dorsalis pars medialis of the telencephalon. Among these first-order and higher-order octaval nuclei, the DON, the secondary octaval population, NC, and CP have been neurophysiologically verified as major auditory nuclei in both hearing specialists and generalists (Echteler 1985a, Lu and Fay 1993, 1995, 1996, Crawford 1993, 1997, Bodnar and Bass 1997, Kozloski and Crawford 1998, 2000).

NEURAL PROCESSING OF SIMPLE SOUNDS

Frequency Coding

There are two theories regarding frequency analysis in vertebrates: frequency selectivity and phase-locking. It is known that sound frequencies are mapped along the basilar membrane in the ears of terrestrial vertebrates (the place code), and this tonotopical organization is

preserved in their brains. However, tonotopical organization is not well presented in the ear and brain of fish (Echteler 1985b, Sento and Furukawa 1987). Compared to auditory afferents of terrestrial vertebrates, fish's auditory afferents are broadly tuned and sensitive to low frequencies (Fay 1988). This low frequency selectivity is consistent with the underwater environment where low-frequency sounds travel longer distances than high-frequency ones. Fig. 2 shows a tuning curve of a saccular afferent, which has its best sensitivity (BS, the lowest threshold) at 60 dB re: 1 µPa, characteristics frequency (CF, the frequency at BS at 200 Hz), and Q_{10dB} of 0.67. Q_{10dB}, a ratio of CF over the frequency bandwidth at 10 dB above threshold, represents frequency selectivity or tuning of auditory neurons. The greater a $Q_{10dB,}$ the stronger the tuning.
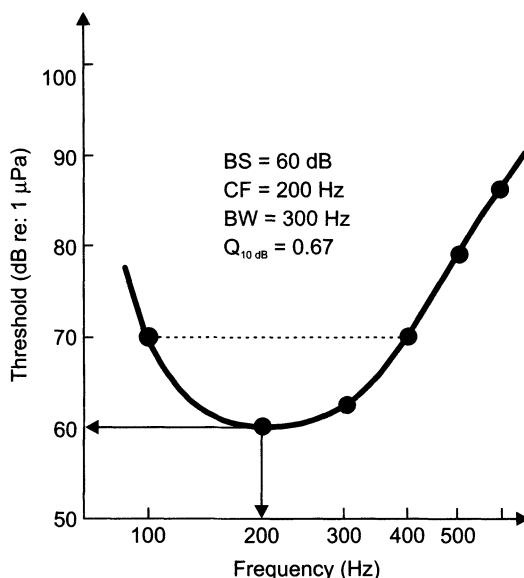
Fig. 2 Schematic drawing of a tuning curve of a saccular afferent, showing best sensitivity – BS, bandwidth – BW, characteristic frequency – CF, and Q_{10dB}.

Furukawa and Ishii (1967) categorized saccular afferents of the goldfish into S1 (250 to 400 Hz) and S2 (700 to 800 Hz) groups based on their CFs. Fay and Ream (1986) re-classified goldfish's saccular afferents into four groups: untuned, low-CF (120 to 290 Hz), mid-CF (300 to 670 Hz), and high-CF (790 to 1770 Hz). In the toadfish, saccular afferents fall into two categories with CFs at 81 Hz and 140 Hz (Fay and Edds-Walton 1997b). Hearing generalists are sensitive to lower frequencies and have a narrower frequency response range than hearing specialists. These differences appear to result from the absence or presence of the Weberian ossicles or gas bladders that are acoustically coupled to the inner ear. Since auditory afferents in fishes are broadly tuned and fall in discrete CF groups, the place code may not play an important role in frequency analysis in fishes.

A few studies of frequency selectivity of auditory neurons in fish's brains have been conducted. Auditory neurons in the torus semicircularis of the goldfish are more sharply tuned

than saccular afferents. The Q_{10dB} of these toral neurons in the goldfish ranges from 2 to 5 while no saccular afferent has its Q_{10dB} greater than 2 (Lu and Fay 1993). Enhancement of frequency selectivity appears to result from inhibition that likely occurs below and/or above CF in the midbrain of the goldfish (Lu and Fay 1993, 1996). Sharply tuned auditory neurons were also found in the midbrain of the mormyrid *Pollimyrus isidorri* (Crawford 1993).

Lu and Fay (1995) found that most auditory neurons in the central posterior nucleus (CP) of the thalamus of goldfish are broadly tuned. Fig. 3 shows examples of response areas and tuning curves of typical saccular, toral, and CP neurons, indicating changes of frequency selectivity at different levels of the auditory pathway. Frequency selectivity is apparently enhanced prior to or at the level of the midbrain, and further sharpening of tuning does not appear to take place in the auditory thalamus. It is likely that the auditory midbrain and thalamus play different roles in hearing. Broad tuning at the auditory periphery is strongly increased in the midbrain, suggesting that the midbrain contributes to frequency domain analytic listening (Bregman 1992). Since most thalamic neurons are broadly tuned with multiple peaked response areas and not phase-
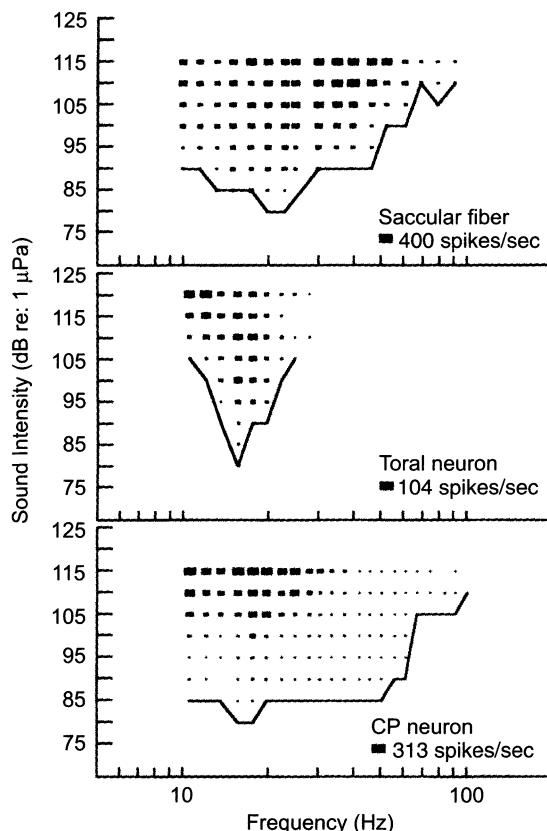
Fig. 3 Response areas and tuning curves of representative saccular afferent, toral neuron, and CP neuron of the goldfish. The size of each black square represents a spike rate. (From Lu and Fay 1995, with permission, © 1995 Springer-Verlag New York Inc.)

locked, the auditory thalamus may be responsible for processing complex sounds with broad spectra, synthetic listening (Bregman 1992).

Most auditory afferent neurons in fishes synchronize to fine structure of tonal stimuli (phase-locking) (Fay 1978a, Moeng and Popper 1985, Fay and Edds-Walton 1997a, Lu et al. 1998, McKibben and Bass 1998, Suzuki et al. 2002). Many of these afferents are able to fire a spike per stimulus cycle, and spikes produced by an afferent are phase-locked at approximately the same phase/time in the stimulus period (see Fig. 4). The temporal information such as inter-spike interval and phase encoded in a train of spikes can be decoded in the brain to extract the stimulus frequency. Coefficient of synchronization (R), ranging from 0 to 1, is often used to represent the strength of phase-locking from no synchronization to perfect synchronization (Goldberg and Brown 1969, Anderson 1973). The strength of phase locking varies with sound frequency and intensity. In most cases, R increases with stimulus intensity. However, some sac-cular afferents are able to fire more than one spike per cycle when presented with tonal stimuli that are lower than 100 Hz. In this case, R calculated based on the stimulus period tends to decrease with stimulus level. Fish's auditory afferents can synchronize to tonal waveforms up to at least 1000 Hz. The strength of phase locking appears to fall along fish's auditory pathways from the periphery to CNS (Fig. 5). This could simply result from an increase in number of chemical synapses in the auditory pathways.
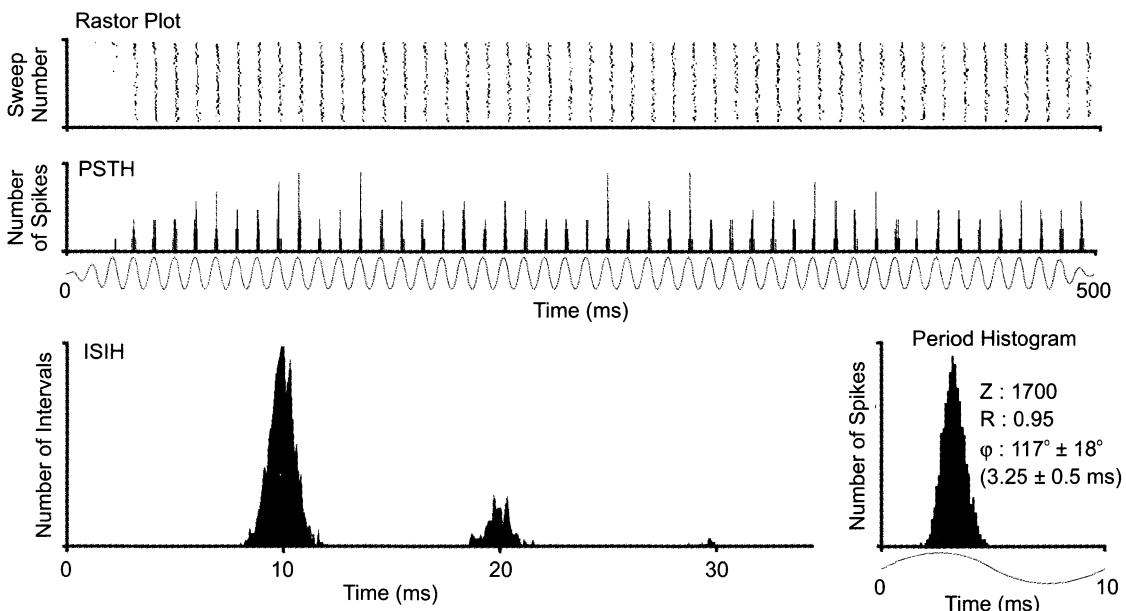
Fig. 4   Raster plot, peri-stimulus-time histogram (PSTH), inter-spike-interval histogram (ISIH), and period histo-gram of a saccular afferent in response to a 100-Hz tone with 500-ms duration and 20-ms rise-and-fall times for 50 repetitions. A sweep of stimulus waveforms is shown under the PSTH. The mean phase of spikes in the period histogram is 117° (or 3.25 ms) with a mean angular deviation of 18° (or 0.50 ms). A cycle of stimulus waveform is shown under the period histogram. Coefficient of synchronization (R) is 0.95, and Rayleigh statistic (Z) is 1700. Z is a combined measurement of coefficient of synchronization and number of spikes ($Z = R^2 \cdot N$, where N is the number of spikes).
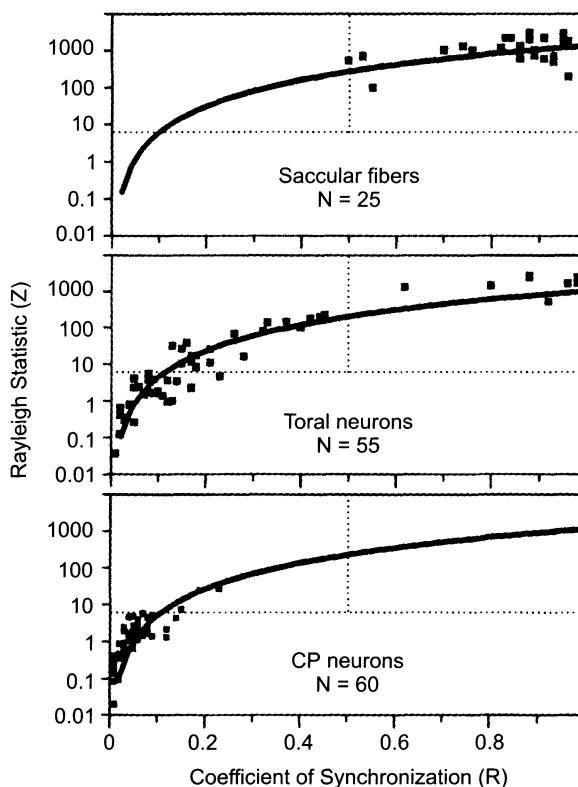
Fig. 5 Comparisons of Rayleigh statistic (Z) versus coefficient of synchronization (R) for saccular afferents, toral neurons, and CP neurons of the goldfish. The *horizontal dotted* lines indicate the critical value, Z = 6.91, dividing neurons into significant phase locking (*above*) and nonphase-locking (*below*). The Z value corresponds to $p = 0.001$. The *vertical dotted* lines, R = 0.5, classify the phase-locked neurons into weakly phase-locked (*upper left*) and strongly phase-locked (*upper right*) neurons. The power-regression curves (*solid*) best fit the data. (From Lu and Fay 1995, with permission, © 1995 Springer-Verlag New York Inc.)

Fay (1978a) showed that behavioral frequency discrimination abilities of the goldfish correlate with temporal errors of phase-locking of its auditory afferents, indicating that phase-locking is the peripheral basis of frequency coding in fish. In Fig. 6, each open square indicates a just-noticeable difference in ms, a psychological error, at a frequency (Fay 1970). The just-noticeable difference is greater at lower frequencies than at high frequencies, and vice versa. Each solid circle or square represents a standard deviation of spike times in a period histogram, a neural error, for a given saccular afferent at 35 dB above the behavioral threshold at a frequency. Standard deviations of spike times for different saccular afferents distribute in a similar range in the logarithmic scale. At a stimulus frequency, the smallest standard deviation of spike times of saccular afferents matches approximately the psychophysical just-noticeable difference.
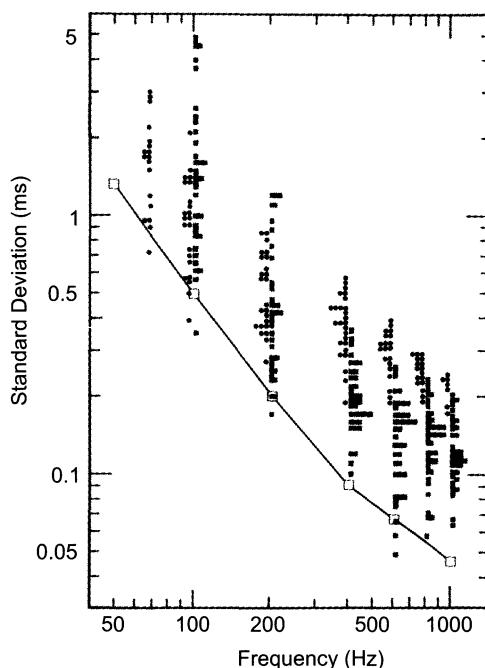
Fig. 6 Psychophysical just-noticeable difference and neural error of phase locking versus frequency for the goldfish. *Open squares* are mean just noticeable differences in stimulus periods using a psychophysical method for four goldfish. *Solid squares* and *solid circles* are temporal jitters for 67 high-CF and low-CF saccular afferents, respectively. Each spike phase from 0° to 360° in a period histogram was converted into a spike time. For a given frequency, a standard deviation was calculated from spike times in a period histogram at 35 dB above the behavioral threshold of the goldfish. (Modified from Fay 1978, with permission, © 1978 Nature Publishing group.)

Temporal Processing

Most fish hearing studies were conducted using simple sounds such as pure tones that consist of single frequencies. Fish's auditory afferents respond to simple sounds in various temporal response patterns (Fay 1978b, Coombs and Fay 1987). Based on the degree of adaptation, temporal response patterns of fish's saccular afferents can be categorized into sustained (little or no adaptation), primary-like (medium adaptation), and onset (high adaptation). These response patterns depend upon several factors such as response properties (spontaneous activity, CF, sensitivity, and tuning) of auditory afferents and physical properties of acoustic signals (frequency and intensity). For example, goldfish's saccular afferents with low-CFs or mid-CFs tend to have sustained or primary–like responses below and at CFs and onset responses above CFs, while untuned and insensitive saccular afferents have an onset response pattern that is independent of stimulus frequency and intensity (Coombs and Fay 1987).

Lu and Fay (1993) found a diversity of temporal response patterns of auditory neurons in the torus semicircularis of the goldfish, including sustained, primary-like, onset, chopper,

pauser, bursting, and build-up. Many of these response patterns resemble those found in the mammalian cochlear nucleus (Rhode and Greenberg 1992). Although some response patterns of auditory toral neurons are similar to those of auditory afferents, others are not observed in the auditory periphery, suggesting that the unique temporal analysis occurs in the central auditory system. For example, chopper neurons discovered in the torus of the goldfish have high regularity of responses to tonal stimuli. They discharge with fixed inter-spike intervals that are independent from stimulus periods. Thus, peripheral temporal response patterns are apparently transformed into central response patterns. Recently, the chopper response pattern found in the goldfish's midbrain was observed in the medulla of the mormyrid *Pollimyrus adspersus*, indicating that this temporal transformation may already start at the level of the medulla (Kozloski and Crawford 2000).

NEURAL PROCESSING OF COMMUNICATION SOUNDS

Pollimyrus adspersus

The mormyrid fish *Pollimyrus adspersus* (Family *Mormyridae*) are hearing specialists because of their high hearing sensitivity (Fletcher and Crawford 2001). The behavioral audiogram of *P. adspersus* shows that they are most sensitive to 500-Hz tones with the corresponding threshold at about 70 dB re: 1 µPa. This species has a pair of gas-filled bladders acoustically coupled to their saccules. Experimental displacement of the gas of both bladders with saline resulted in significant threshold increases, up to 30 dB, in the frequency range from 100 to 3000 Hz, indicating that the bladders play a considerable role in sound detection. *P. adspersus* live in murky water in the Niger River of West Africa, and they use both acoustical and electrical signals in communication. This review only focuses on how their acoustic signals are processed in the midbrain. Male *P. adspersus* court females with acoustic displays that start with an alternating sequence of moans and grunts and finish with a growl (Fig. 7A; see Crawford et al.1986, 1997). These courtship sounds (moans, grunts, and growls) are composed of clicks with inter-click intervals from 5 to 50 ms.

Crawford (1997) made single-cell recordings from auditory neurons in nucleus medialis dorsalis of the midbrain in response to click trains with various inter-click intervals that resemble those of courtship sounds. He found that about 30% of the toral neurons recorded were inter-click-interval selective (see Fig. 7B). For example, the neuron shown in Fig. 7C is most selective to the inter-click interval of 18 ms. Interval selectivity appears to be unique neural computations in the midbrain because interval selective neurons are not observed in the medulla of this species (Kozloski and Crawford 1998). In general, a group of auditory midbrain neurons of *P. adspersus* are temporally tuned to different inter-click intervals, suggesting that these neurons are able to extract acoustic temporal information of the signals that resemble the species' natural sounds. Therefore, the auditory midbrain of *P. adspersus* contributes to detecting conspecific communication sounds.
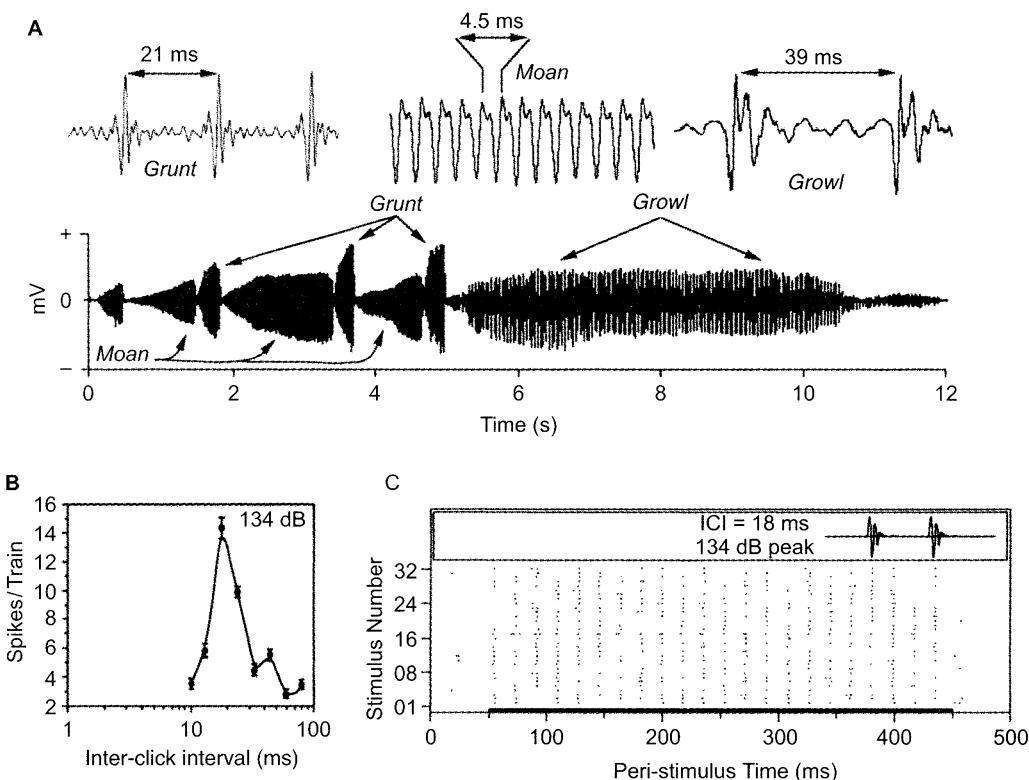
Fig. 7 (A) Courtship sounds produced by a male *P. adspersus*. (B) Spike rate versus inter-click interval (ICI) for an auditory midbrain neuron of *P. adspersus*. Stimulus level: 134 dB re: 1 µPa (peak-to-peak). (C) Raster plot of spike times recorded from the same neuron shown in B in response to 32 stimulus presentations. The *solid horizontal* bar indicates the duration of the click train, starting at 50 ms and ending at 450 ms. (Modified from Crawford 1997, with permission, © 1997 Springer-Verlag New York Inc.)

Porichthys notatus

The midshipman fish *Porichthys notatus* (Family *Batrachoididae*) inhabit deep coastal waters of western North America in winter and migrates to shallow intertidal and subtidal zones to breed in the late spring and summer. Midshipman are polymorphic, having one female and two types of male reproductive morphs. Type I males, on average about 50% larger than type II males and females, build nests and produce advertisement calls ("hums") to attract gravid females to their nests. Dimorphic males are distinguishable by a suite of behavioral, somatic, neurobiological and endocrinological traits (Bass 1992, 1996 for reviews).

Type I male hums are continuous, multiharmonic sounds lasting from a few seconds to 60 min, with a fundamental frequency close to 100 Hz in its natural habitat though it varies with ambient temperature (Fig. 8A) (Ibara et al. 1983, Brantley and Bass 1994, Bass et al. 1999). When two pure tones (F1 and F2) with a small difference in frequency are added together, the result is a beating stimulus that is amplitude-modulated at the rate of (F2-F1)/2. Humans

perceive the beating stimulus as a tone of (F1+F2)/2 with the loudness changes at the rate of the difference between the two frequencies (dF). In the natural habitats of the midshipman, a male's hum (F1, fundamental frequency) is often modulated by a hum (F2) produced by another male nesting nearby, resulting in a concurrent signal or beat (Fig. 8B). Females have to extract each hum from the acoustic beat in order to decide which hum is more attractive and then approach that sound source (McKibben and Bass 1998). Bass and his colleagues investigated peripheral and central neural mechanisms underlying the detection of the concurrent hum. They found that saccular afferents are more sensitive to individual hums (F1 and F2) instead of the beat frequency (dF) (Fig. 8C) (McKibben and Bass 2001). In contrast, auditory neurons in NC are more selectively sensitive to beat dF rather than individual hums (Fig. 8D) (Bodnar and Bass 1997). For the midshipman, the peripheral code of fundamental frequencies of individual hums is apparently transformed into the central code of the frequency difference
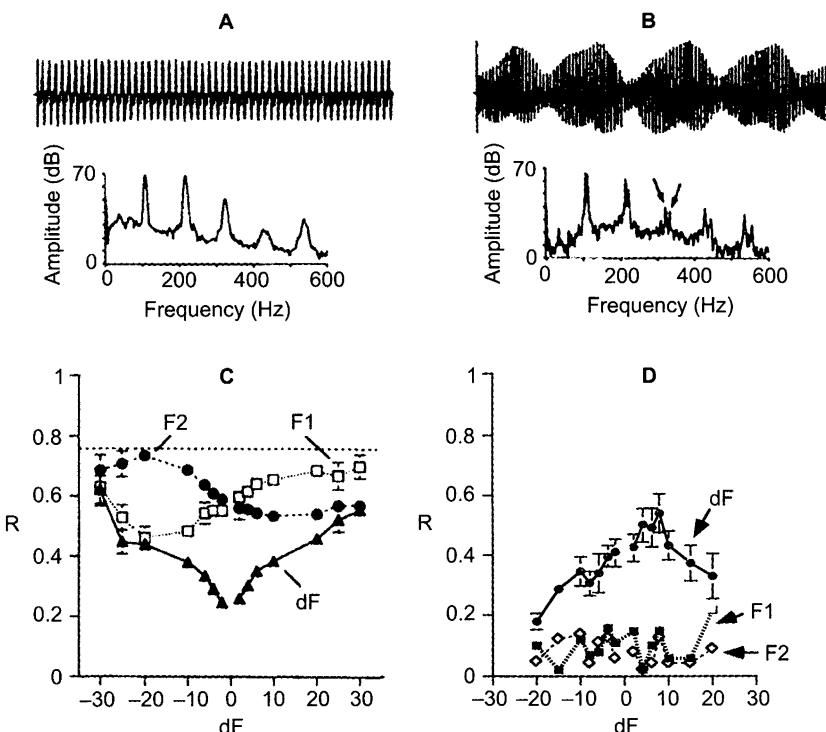
Fig. 8 (A) Waveform and spectrum of a hum produced by a Type I male *P. notatus*. (B) Waveform and spectrum of a concurrent hum with F1 = 90 Hz and F2 = 100 Hz. The two *arrows* indicate separated peaks of third harmonics of F1 and F2. (C) Mean R (± standard error) versus dF for 35 saccular afferents with F1 fixed at 90 Hz and variable F2. R was calculated from three different period histograms with periods of 1/F1 (*open squares*), 1/F2 (*filled circles*), and 1/dF (dF = F2 − F1, *filled triangles*). The *dashed* line indicates mean R when F1 was presented alone (Mean ± SD: 0.76 ± 0.19). (D) R versus dF for a typical toral neuron that is selectively tuned to a specific dF of about 8 Hz. F1 was fixed at 90 Hz while F2 varied in a range from 70 to 110 Hz. (A to C, modified from McKibben and Bass 2001, with permission, © 2001 Springer-Verlag New York Inc.; D, modified from Bodnar and Bass 1997, with permission, © 1997 Society for Neuroscience.)

between them. Since both DON and the secondary octaval population converge on NC, they likely contribute to this transformation of neural coding of the concurrent hum (Bass et al. 2000).

DIRECTIONAL HEARING

Sound localization is one of the most important functions of auditory systems of vertebrates, most of whom, including fishes, live in dim/dark habitats and rely on their auditory systems to find or avoid sound sources (prey or predators). It is known that terrestrial vertebrates localize a sound source using binaural cues and pinna filtering. They determine the azimuthal direction of a sound by comparing timings and intensity of acoustic information received by two ears. It is also clear that pinna filtering is crucial for sound localization in elevation, particularly the mid-sagittal plane, where there are no binaural time and intensity differences (Batteau 1967). Terrestrial vertebrates have greater localization capacities in the horizontal plane than eleva-tion, which is consistent with the two-dimensional habitat where they live.

However, the cues used by terrestrial vertebrates are not likely available for fishes living in the underwater environment because of the following reasons. First, sound travels about four to five times faster in water than in air. Second, the distance between two ears of a fish is very small, about 5 mm for most fishes, and the two ears are acoustically coupled to each other. These factors result in minimal binaural cues, which appear to be useless for fish to localize a sound source. In addition, fish do not have the external ear as used by terrestrial vertebrates for sound localization in elevation. Therefore, mechanisms of sound localization by terrestrial vertebrates do not appear to apply to fishes.

Although data on sound localization by fishes are limited, previous behavioral studies showed some evidence of the ability of fishes to localize a sound source (see Fay and Feng 1987 for a review). Early experiments demonstrated that the carp (*Cyprinus carpio*), goldfish, and squirrelfishes (*Myripristis berndti* and *M. argyromus*) are able to turn away or toward a sound source (Moulton and Dixon 1967, Kleerekoper and Malar 1968, Popper et al. 1973). Intensive studies on the cod (*Gadus morhua*) revealed that it is also able to orient toward a sound source (Schuijf 1975). The cod can detect a change in direction of sound propagation, and discrimi-nate two spatially separated sound sources in both the horizontal and mid-sagittal planes (Chapman and Johnston 1974, Hawkins and Sand 1977). Humans have poor directional detec-tion capabilities in the mid-sagittal plane (Gardner and Gardner 1973), but the cod has equiva-lent discrimination capabilities (10° to 20°) in both the horizontal and the mid-sagittal planes (Chapman and Johnston 1974, Hawkins and Sand 1977). In addition, the cod can discriminate two sound sources that are positioned at different distances (Schuijf and Hawkins 1983).

The question of how fish localize a sound source has been puzzling researchers since von Frisch (1938). Among several hypotheses of mechanisms underlying sound localization by fishes bearing a swim bladder (Schuijf 1976, Schellart and Munck 1987, Popper et al.1987), the dominant one is the phase model proposed by Schuijf (1976), stating that fish localize a sound source by the following two processes: (1) determination of the axis on which the sound

propagates, and 2) determination of the actual direction by analyzing the phase/time difference between particle motion and pressure inputs to the brain. This hypothesis has been supported by some experimental results. Field behavioral studies have demonstrated that the cod can distinguish two sound sources that are separated by 180° in the horizontal and mid-sagittal planes (Schuijf 1975, Schuijf and Buwalda 1975). In addition, Buwalda et al. (1983) provided convincing evidence that the cod is able to detect the phase difference between particle motion and pressure stimulations. Rogers et al. (1988) extended the phase model to localization of complex sounds as well as pure tones. Other hypotheses for sound localization by fishes without a swim bladder were proposed by Corwin (1981) and Kalmijn (1988), and recently reviewed by Myrberg (2001).

Peripheral Structure Basis of Directional Hearing

Sensory hair cells are mechanoreceptors found in the auditory and vestibular organs of all vertebrates as well as in the lateral line systems of fishes and some amphibians (Lewis et al. 1985). The structural asymmetry of hair cell cilia defines the morphological polarization. It has been hypothesized that fish determine the directional information, the axis at which a sound wave travels, using arrays of spatially oriented hair cells in the otolithic organs (Schuijf and Buwalda 1975, Popper 1976). The morphological and physiological polarity of hair cells provides the peripheral basis for directional hearing in fish. Data of morphological polarization patterns of sensory hair cells in the otolith organs have been obtained in many fish species, including sound-producing fishes such as the cod, toadfish, and catfishes, and those that do not vocalize such as the goldfish and sleeper goby (Dale 1976, Popper 1977, Platt 1977, Popper 1981, Saidel et al. 1990, Edds-Walton and Popper 1995, Lu and Popper 1998, and Ladich and Popper 2001).

To determine morphological polarizations of hair cells, it is necessary to visualize stereocilium and kinocilium locations in the ciliary bundle of a hair cell. In contrast to using traditional scanning electron microscopy, morphological polarity patterns of sensory hair cells in the otolithic organs were mapped using immunocytochemical and confocal imaging technologies (Lu and Popper 1998). This method allows us to rapidly determine hair cell polarization patterns and is an important complement to studies on the correlation between morphological polarity of hair cells and response directionality of physiologically characterized afferent neurons that innervate the hair cells. Fig. 9 shows orientation patterns of hair cells in the saccule, lagena, and utricle of the sleeper goby. In addition to the diversity of hair cell orientation patterns, otolithic organs of the sleeper goby and other fishes have different spatial orientations. For example, the saccular epithelium of the sleeper goby is positioned perpendicular to the horizontal plane and deviates about 40° from the fish's mid-sagittal plane. The lagenar epithelium is also perpendicular to the horizontal plane but deviates about 16° off the mid-sagittal plane. The utricle lies on the horizontal plane. Thus, the sleeper goby's ear as well as those in other fish species is morphologically capable of being a 3-D sound detector (Lu and Popper 1998).
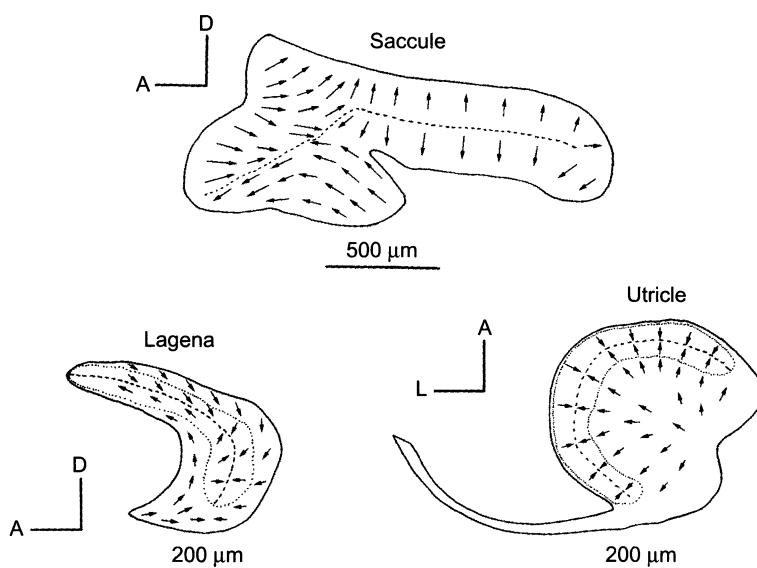
Fig. 9 (A) Orientation patterns of hair cells in the saccule, utricle, and lagena of the sleeper goby. *Arrows* point to the morphological polarizations of hair cells in these sensory epithelia. The *dashed* lines in these epithelia indicate the polarity transition lines. The areas highlighted by *dots* in the lagenar and utricular epithelia are striolar regions. *A* anterior, *D* dorsal, and *L* lateral. (Modified from Lu and Popper 1998, with permission, © 1998 Elsevier Science B. V.)

Directional Coding of Auditory Afferents

Single–cell recordings from saccular afferents in response to acoustic particle motion have been conducted on several teleost species: the cod, goldfish, toadfish, and sleeper goby (Hawkins and Horner 1981, Fay 1984, Fay and Edds-Walton 1997a, b, Lu et al. 1998, Edds-Walton et al. 1999, Lu and Popper 2001, Weeg et al. 2002). Results from these studies have shown that saccular afferents are sensitive to linear accelerations, and most sensitive saccular afferents in both hearing specialists and generalists have a similar displacement threshold, 0.1 to 0.2 nm. This displacement threshold is consistent with the vibrational sensitivity of saccular and lagenar afferents of the bullfrog (Koyama et al. 1982). The behavioral detection threshold in a hearing generalist, the oscar (*Astronotus ocellatus*), is about 1 nm in displacement (Lu et al. 1996).

In addition, almost all saccular afferents in the fish species studied are directionally selective. Fig. 10 shows an example of a neurophysiologically characterized and neuroanatomically identified saccular afferent neuron whose dendritic terminals innervate hair cells in the middle region of a saccular epithelium (Fig. 10A and B). Directional response properties of the saccular afferent neuron were characterized using the whole-cell recording technique, and then the neuron was filled with a neuronal tracer, Neurobiotin. The best response axis, the mean angle computed based on the data points at six stimulus axes, of this saccular neuron is about 106° (Fig. 10E). The average morphological polarity of the hair cells innervated by the saccular afferent was later determined using the immunochemical and confocal microscopy
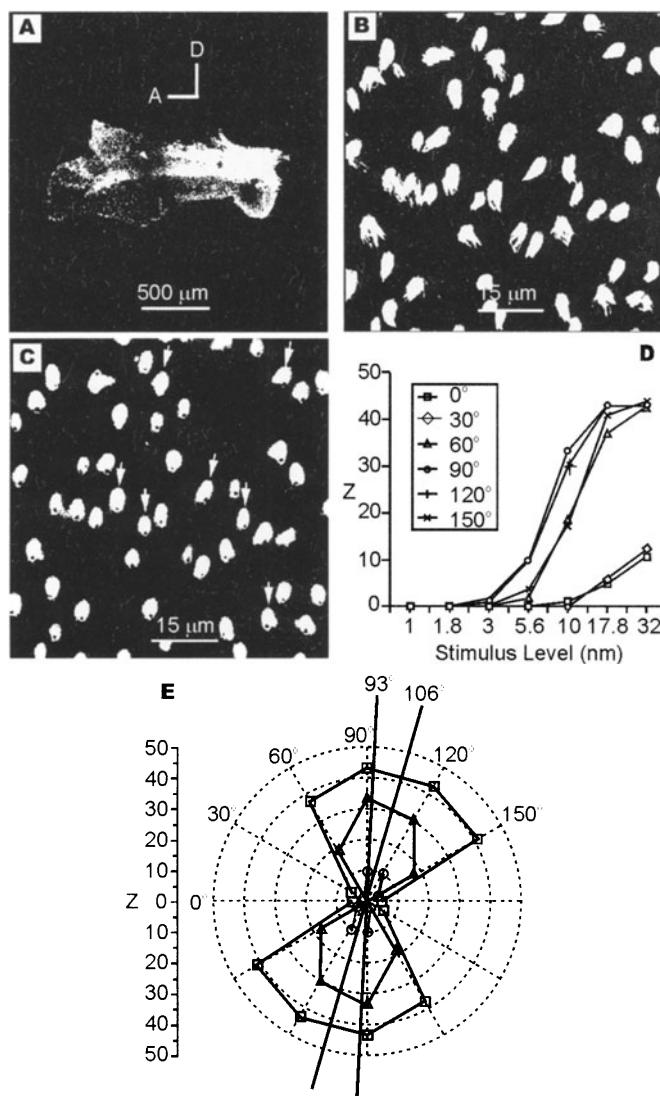
Fig. 10 Morphophysiology of a saccular ganglion neuron of the sleeper goby. (A) Confocal image of a whole mount of saccular epithelium. The dendritic arbor of a Neurobiotin-filled saccular neuron was labeled with Texas red, and hair cell ciliary bundles were stained with Oregon green phalloidin that tags stereocilia, but not kinocilia, of hair cell ciliary bundles. The image orientation is the same for A, B, C, and E. The *arrowhead* indicates the location of dendritic terminals of the filled sacular neuron. *A* anterior, and *D* dorsal. (B) 3-D Image of double labeling of ciliary bundles of hair cells and dendritic terminals of the neuron. (C) Single optical section from the 3-D image of ciliary bundles shown in B. *Light ovals* are stereocilia of ciliary bundles of hair cells. The *dark circle* at one end of each ciliary bundle is the location of unlabeled kinocilium. The *arrows* indicate morphological polarizations of seven hair cells innervated by the dendritic arbor of the saccular afferent neuron. (D) Z versus stimulus level functions of the saccular neuron at six stimulus axes in a vertical plane that is parallel to the saccular epithelium ($0°$ is the longitudinal axis of the epithelium, and $90°$ is its dorsoventral axis). (E) Z versus stimulus angle functions at three different stimulus levels (5.6 nm, *circles*; 10 nm, *triangles*; 17.8 nm, *squares*). The solid line at $106°$ represents the best response axis of the saccular neuron while the solid line at $93°$ indicates the average morphological polarity of innervated hair cells. Stimulus frequency: 100 Hz. (Modified from Lu and Popper 2001, with permission, © 2001 Springer-Verlag New York Inc.)

methods. Confocal imaging revealed that dendritic terminals of the saccular ganglion neuron innervate 14 hair cells with their morphological polarizations in a range from 87° to 115° (mean = 93° ± 6°) (see Fig. 10C). The saccular neuron has typical monotonic Z-level functions, showing the lowest threshold at about 3 nm (Fig. 10D). Neural response directionality of the saccular afferent is consistent with the average morphological polarity of innervated hair cells.

Twenty nine saccular afferent neurons in the sleeper goby were directionally characterized, completely filled, and clearly stained after immunochemical processing (Lu and Popper 2001). Fig. 11A summarizes innervation locations, in saccular epithelia, of these neurons. These innervation loci distribute across a large proportion of the epithelium except for the ventral region of the rostral part of the epithelium. Fig. 11B shows the structure-function relationship between
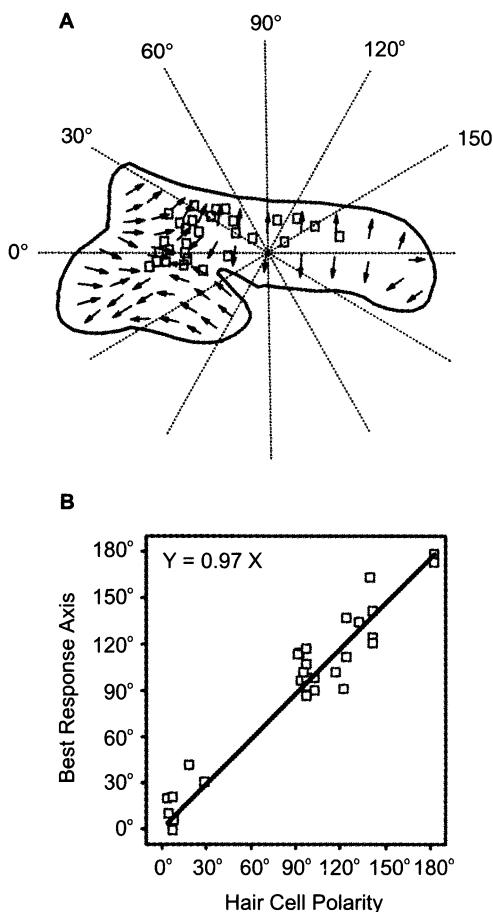
Fig. 11 (A) Typical orientation map of hair cells in the saccule of the sleeper goby and innervation loci (*open squares*) of dendritic terminals of 29 saccular ganglion neurons. The *arrows* show morphological polarizations of hair cells while the *dashed* lines indicate stimulus axes. (B) Correlation between best response axes of saccular ganglion neurons and morphological polarizations of the hair cells innervated by the saccular neurons. (Modified from Lu and Popper 2001, with permission, © 2001 Springer-Verlag New York Inc.)

response directionality of the saccular neurons and morphological polarity of innervated hair cells, illustrating a significant correlation between them ($r = 0.97$). It is also noted that no saccular neuron was found to have its best response axis at 60°. This is consistent with the observation that there are few, if any, hair cells oriented at this axis.

Directional Processing in the Brain

We do not know much about central auditory processing of directional information of a sound source because only a few studies have been reported (Wubbels and Schellart 1997, Fay and Edds-Walton 1999, Ma and Fay 2001). Wubbels and Schellart (1997) studied neural encoding of sound direction in the torus semicircularis of the trout (*Oncorhynchus mykiss*) in response to directional stimuli in the horizontal plane. They found that about 45% of the neurons recorded in the torus were directionally sensitive and 75% of these neurons were phase-locked to tones. In addition, they observed directionally sensitive neurons in the medial torus were "tuned" to the rostrocaudal axis while neurons in the lateral part had various best response axes. In general, directionality of toral neurons in the trout is topographically organized though the topographical map is not as well organized as those in other vertebrates (Knudsen and Konishi 1978, Middlebrooks and Knudsen 1984).

Investigations of directional response properties were conducted on auditory neurons in the DON of the toadfish (Edds-Walton and Fay 1998, Fay and Edds-Walton 1999, Fay and Edds-Walton 2000). They found that most neurons recorded were directionally sensitive and phase-locked to pure tones and many of these neurons appeared to be more directionally selective than auditory afferents. Fay and Edds-Walton (1999) suggested that inhibition could account for the sharpness of directional selectivity in the medulla of the toadfish. Similar findings were also observed in the torus of the goldfish (Ma and Fay 2001).

Role of the Saccule in Directional Hearing

The saccule is the otolithic organ that has been most frequently studied in directional hearing in fishes. Previous neurophysiological studies reported that saccular afferents are directionally sensitive to acoustic particle motion (Fay and Edds-Walton 1997a, Lu et al. 1998) and that neural response directionality derives specifically from the polarization of saccular hair cells that are innervated by them (Lu and Popper 2001). However, it is not clear what role the saccule contributes to overall directional sensitivity. The saccular sensory epithelium in the sleeper goby is positioned perpendicular to the horizontal plane and deviates 40° from the mid-sagittal plane of the fish, indicating that the saccule may be important in both elevational and azimuthal localization (Lu and Popper 1998).

Using auditory brainstem recording techniques, Lu and Xu (2002) revealed the role of the saccule in directional hearing sensitivity by comparing auditory thresholds of normal sleeper gobies to those with unilateral/bilateral removal of saccular otoliths. The goby has different hearing thresholds in either the horizontal plane (2–3 nm, one-way ANOVA, $p < 0.05$) or mid-sagittal plane (1–2 nm, $p < 0.05$) (see Fig. 12). In the horizontal plane, removal of the right

saccular otolith resulted in 4 to7 dB decreases in hearing sensitivity at $-90°$, $+30°$, and $+60°$, but no significant changes at other axes. In the mid-sagittal plane, unilateral saccular removal did not significantly decrease sensitivity at any axis except $0°$. In both the horizontal and mid-sagittal planes, bilateral removal of saccular otoliths resulted in robust hearing loss of 28 to 32 dB
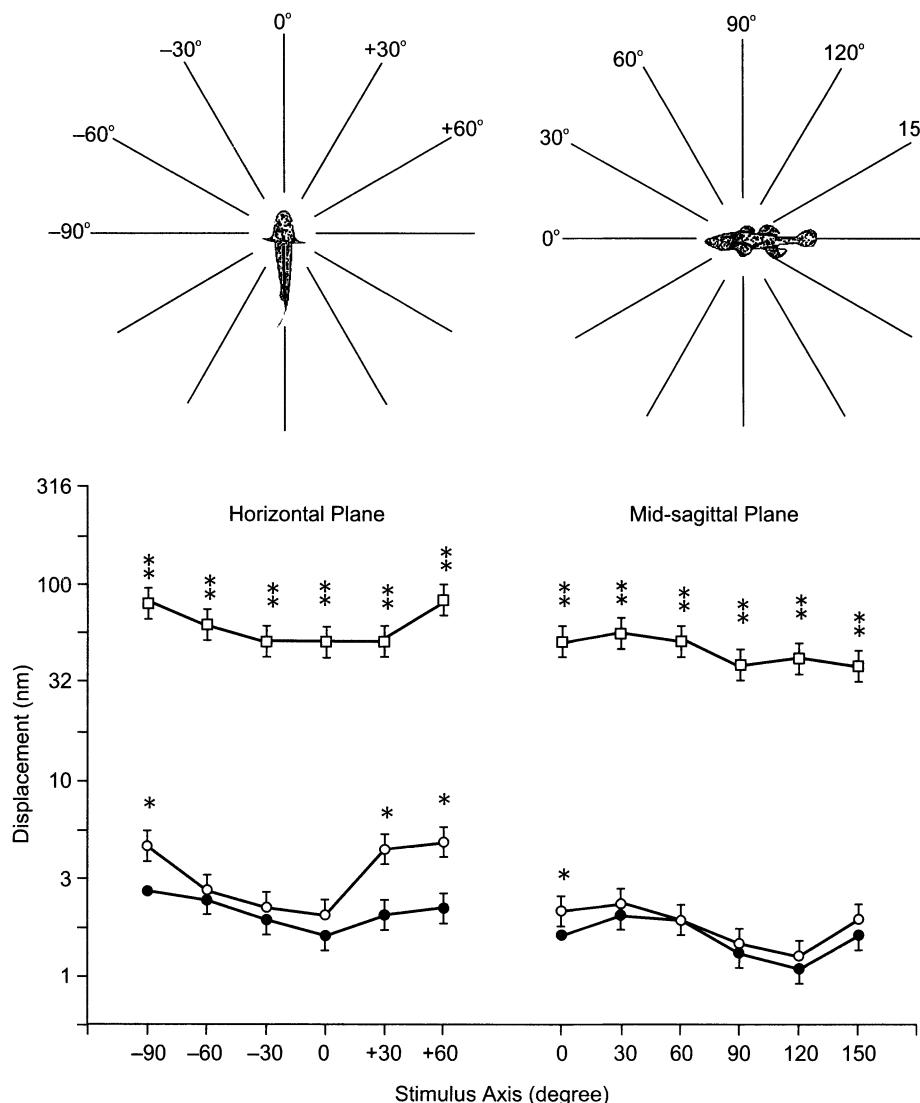
Fig. 12 *Top panel*: Stimulus axes in the horizontal and mid-sagittal planes. *Bottom panel*: Auditory thresholds of the sleeper goby. *Solid circles*: normal thresholds, *open circles*: thresholds with removal of the right saccular othlith, and *open squares*: thresholds with removal of both saccular otoliths. *: Significant difference between control and experimental thresholds (one-tailed Student's *t*-test, $p < 0.05$), and **: Strongly significant difference between control and experimental thresholds ($p < 0.001$). N = 6. (Modified from Lu and Xu 2002)

at all axes. Therefore, the results indicate that in the horizontal plane the saccule appears to be most sensitive to detect acoustic stimulation at the axis that is consistent with its azimuthal orientation at $+40°$ or $-40°$, and that unilateral loss of saccules cannot be compensated by the other saccule, or other otolith organs. However, unilateral saccular otolith removal does not significantly alter hearing sensitivity at most stimulus axes in the mid-sagittal plane, indicating that other otolith organs may play complementary roles in elevational detection. In general, the goby's saccule plays important roles in directional hearing in both the horizontal and mid-sagittal planes.

Acknowledgements

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Neuroethology and Sensory Ecology of Teleost Ultrasound Detection

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ABSTRACT

Recent discoveries have shown that some species of fish can detect frequencies as high as 180 kHz. This is well above what had been thought to be the limit of fish detection abilities but it remains unclear exactly how fish are performing this ultrasound detection. One obvious selective mechanism for evolution of ultrasound detection by fishes is predation by echolocating odontocetes. Little evidence is available to either accept or refute this hypothesis however. The current chapter reviews the evidence for ultrasound detection in fish, both in the field and under laboratory conditions. Possible adaptations responsible for this detection ability are reviewed, with special emphasis on the morphology of the auditory system of fishes in the order Clupeiformes (the group most associated with ultrasound detection). The current state of our knowledge of the phylogenetics of the Clupeiformes does not allow definite testing of evolutionary hypotheses in this group but the current hypotheses concerning evolution of ultrasound detection in clupeiform fishes are discussed. The ecological consequences of ultrasound detection are stressed throughout the chapter, especially with regards to the behavioral ecology of predator: prey interactions as they may relate to odontocetes preying upon fish. Future experiments are encouraged that 1) examine how widespread is the ability to detect ultrasound, 2) assess the actual survival advantage of ultrasound detection in response to dolphin predation, 3) determine the precise mechanism responsible for ultrasound detection, and 4) examine the developmental timing of ultrasound detection. It is not until these questions are answered that we can obtain a true picture of the evolution of ultrasound detection in teleosts.

Key words: Ultrasound, Clupeiformes, Gadiformes, Hearing, Sensory Ecology, Evolution

INTRODUCTION

For many years it had been established theory that the upper frequency limit to teleost hearing was *approximately* 5 kHz. In the early 1990s, however, field reports (Nestler et al. 1992; Ross et al. 1993) began to appear indicating that some teleost species showed evasive maneuvers in response to sounds well above this 5 kHz limit. It was initially thought that these fish were not responding to these very high frequencies but instead to some lower frequencies embedded in

the signal. When some of these fish were eventually brought into the laboratory and tested using carefully controlled sound stimuli, indeed some did respond to frequencies well into the ultrasonic range (Astrup and Møhl 1993; Mann et al. 1997; 1998). It has been argued for at least some species that the sensory modality responsible for detection of ultrasound is the auditory system (i.e. at least some species of fish could indeed hear well above what was previously thought to be the limit of their detection). The purpose of the current chapter is to review the evidence for detection of ultrasound in teleost fishes from an ecological context, analyze the potential mechanisms for this detection, and discuss the evolutionary hypotheses that have been put forth to explain detection of ultrasound in the order Clupeiformes. This chapter represents an updating and extension of the fine review done by Astrup (1999) and I encourage the reader to examine that paper for a slightly different perspective.

Before discussing the scientific evidence for ultrasound detection by teleosts, a definition of the term is in order. "Ultrasound" by definition is an arbitrary and anthropomorphic term. It simply refers to sound waves at a frequency above the maximum frequency detectable by human ears (20,000 Hz). There are many species of mammals (e.g. bats; Popper and Fay 1995; cetaceans; Dolphin 2001) and insects (reviewed in Pollack 1998; Fullard 1998) that can hear well above the human maximum of 20,000 Hz and they use a variety of structural adaptations to accomplish this task. Rather than reviewing these studies I direct the readers attention to those reviews cited above. While the current review does use the anthropomorphic definition of ultrasound, what is of especial interest are those instances in which fish can hear up in the 100's of kilohertz range. This represents more than a ten-fold increase in what had been thought of as the upper frequency limit of fish hearing and appears to be restricted to a very few species, based on current findings. The number of species found with sensitivity to ultrasound may increase as this topic receives more interest. All mention of "dB" in the current review refers to dB re 1 μPa.

EVIDENCE FOR ULTRASOUND DETECTION IN FISH

While there is one report of ultrasound detection in goldfish [*Carassius auratus*, up to 50 kHz, (Offutt 1968)] it is not clear if this was truly ultrasonic detection or perhaps was due to other frequencies within the stimulus. None of the other very numerous studies on goldfish hearing have reported responses much above 5 kHz (reviewed in Fay 1988; also see Yan et al. 2000; Higgs et al. 2002; Yan, this volume). All other reports of detection of ultrasonic frequencies have been either in cod (*Gadus morhua*, Gadidae) or fishes in the order Clupeiformes (see Table 1). The two studies done on the cod (Astrup and Møhl 1993; 1998) both used classical conditioning responses to determine the thresholds of cod to a 38 kHz stimulus. Cod show positive responses to a 38 kHz stimulus with a threshold of approximately 195 dB (Fig. 1; Astrup and Møhl 1993), and can distinguish between low and high stimulus repetition rates using a 38 kHz signal (Astrup and Møhl 1998).

Studies of ultrasound detection in clupeoid fishes have been conducted either in the field, using net pens or fish around hydropower intakes, or in the laboratory with behavioral or physiological measures. In field conditions, blueback herring (*Alosa aestivalis*, Clupeidae) show avoidance responses to a 110-140 kHz transducer at 180 dB (at 1 m) in net pens and to a 124-131 kHz

Table 1 Summary of hearing abilities, auditory morphology, and ecology of species tested for ultrasound hearing abilities. Under "type of test" B indicates a free-swimming behavioral trial, CC indicates classical conditioning, and P indicates a physiological measure (auditory brainstem response). Hearing data for shad, menhaden, anchovy, and sardines are from Mann et al. (2001). Hearing data for blueback herring are from Nestler et al. (1992), for alewife are from Ross et al. (1993), and for cod from Astrup and Møhl (1993). Alewife and blueback herring are listed as "not eaten by dolphins" due to their freshwater habitat. Designations as "probably prey" are based on occurrence of related species in gut contents in Barros and Wells (1998) or Würtz and Marrale (1993). The "yes" for menhaden is based on the documented presence of menhaden in the gut contents of *Tursiops truncatus* (Barros and Wells,1998).

Species	Maximum frequency of response (kHz)	Ultrasound threshold (dB)	Type of test	Swimbladder extensions	Adult habitat	Eaten by dolphins?
Blueback herring	140	150	B	Bullae	Freshwater	No
Alewife	128	150	B	Bullae	Freshwater	No
American shad	180	160	CC, P	Bullae	Saltwater	Probably
Gulf menhaden	80	180	P	Bullae	Saltwater	Yes
Bay anchovy	4	—	P	Bullae	Estuarine	Probably
Scaled sardine	4	—	P	Bullae	Saltwater	Probably
Spanish sardine	4	—	P	Bullae	Saltwater	Probably
Cod	34	195	CC	"horns"	Saltwater	Probably

transducer with a source level of 187-200 dB in free field conditions (Nestler *et al.* 1992). Alewives (*Alosa pseudoharengus*, Clupeidae) show avoidance in free field to a 122-128 kHz transducer with a source level of 190 dB (Ross et al. 1993; 1996). The avoidance response was still effective in free field up to 60 m away in blueback herring (Fig. 1; Nestler et al. 1992) and 80 m away in alewives (Fig. 1; Ross et al. 1993; 1996). In the laboratory, American shad (*Alosa sapidissima*, Clupeidae) have been shown to be sensitive to pure tones up to 180 kHz using both classical conditioning (Mann et al. 1997; 1998) and brainstem recordings (Mann et al. 2001) with a threshold of 140-160 dB for ultrasonic tones (Fig. 1). Gulf menhaden (*Brevoortia patronus*, Clupeidae) also respond to pure tones up to 80 kHz, although they are not as sensitive as shad (Fig. 1; Mann et al. 2001). Vigorous behavioral responses have also been found in American shad in response to pure tones from 20-150 kHz, with behavioral response dependent on tone intensity (Plachta and Popper, 2003). As tone intensity increases shad initially form tighter schools and then, as intensity continues to increase, schools break up and fish swimming speed increases dramatically (Plachta and Popper, 2003). In a mixed lab and field study, Pacific herring (*Clupea pallasii*, Clupeidae) showed vigorous behavioral responses to a simulated broadband echolocation call (Wilson and Dill 2002) but this call had significant energy in the sonic range (down to 1.3 kHz). Thus it is not clear that the herring were responding to ultrasound in the Wilson and Dill (2002) study. Interestingly, herring do not respond to outputs from more narrow-band ultrasonic sources (Schwarz and Greer 1984; Wilson and Dill 2002). This suggests that herring cannot detect ultrasonic tones but do clearly respond to cetacean
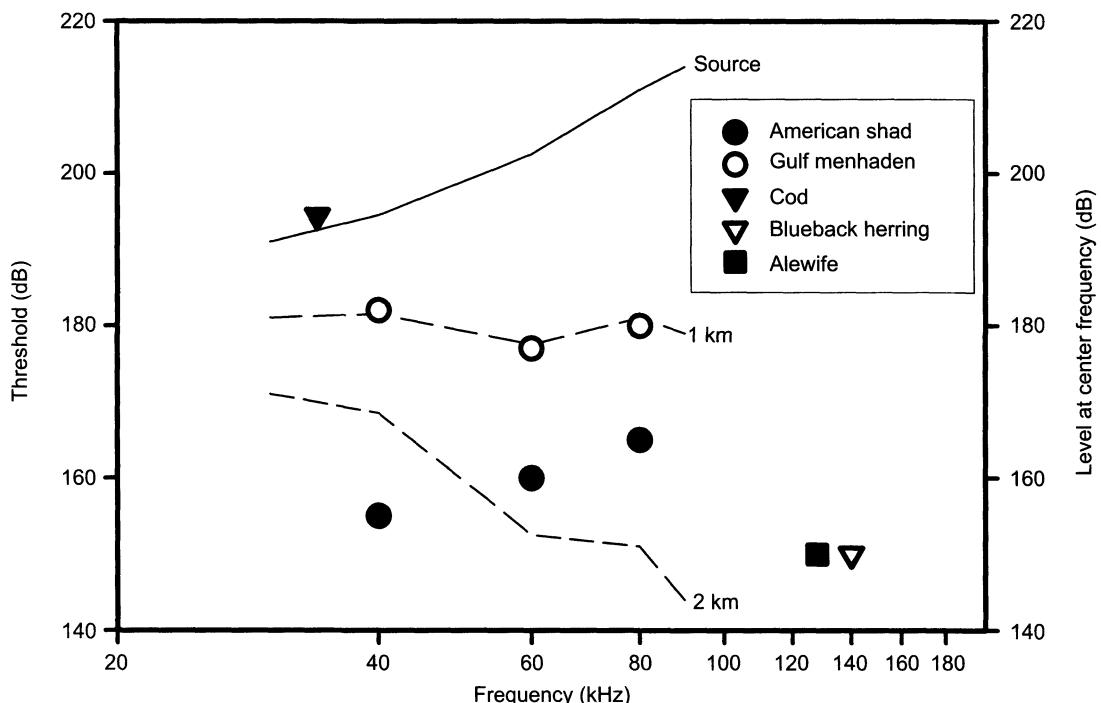
Fig. 1 Auditory thresholds (symbols; left abscissa) for all known species of ultrasound-detecting fish in comparison to output level of the center frequency (lines; right abscissa) of echolocation calls of false-killer whales (*Pseudorca crassidens*; source levels from Au et al. 1995). Distances next to lines are estimates of level at source 1 km away, and 2 km away, based on spherical spreading and ideal absorption of Rogers and Cox (1988). American shad, gulf menhaden, blueback herring, and alewife are all in the clupeid subfamily Alosinae. God are in the Gadidae. Data for shad and menhaden from Mann et al. (2001). Data for blueback herring are from Nestler et al. (1992), for alewife are from Ross et al. (1993), and for cod from Astrup and Møhl (1993).

echolocation calls if emitted over a broad frequency band. Some other clupeoids that have been specifically tested for ultrasound detection have also shown no response. Bay anchovy (*Anchoa mitchilli*, Engraulidae), Spanish sardine (*Sardinella aurita*, Clupeidae) and scaled sardine (*Harengula jaguana*, Clupeidae) were all tested with the same apparatus as the shad and menhaden and their maximum frequency of detection is 4 kHz (Mann et al. 2001). Thus the only fish in the order Clupeiformes with clear evidence for ultrasound detection are those in the subfamily Alosinae (Fig. 1).

ECOLOGICAL IMPORTANCE OF ULTRASOUND DETECTION

In those species that can detect ultrasound the most immediate question is to what are they listening? It has been hypothesized that fish hearing did not evolve to detect specific signals but rather to do a sort of "auditory scene analysis" wherein fish try to get some information on the auditory scene by segregating signals of interest from signals found in the background noise (e.g.

Fay and Popper 2000). This does not seem to be a good strategy for issues of ultrasound detection. Ambient non-biological sources of noise in the ocean and large lakes, where ultrasound-detecting fish are typically found, does not have much energy above about 10 kHz (Wenz 1964). While some of the Crustacea, most notably snapping shrimp (Crangonidae), can produce large amounts of background noise, their limits of output are around 15 kHz (Fish 1964), not high enough to account for ultrasonic detection by cods and clupeoids. The only significant source of natural ultrasound of which I am aware is that emitted by odontocetes in the form of echolocation signals emitted during hunting of prey. Others (Astrup and Møhl 1998; Mann et al. 1998; 2001) have also speculated on the importance of odontocete echolocation as an auditory stimulus. While echolocation is a complex process involving emission of ultrasonic frequencies, reception of reflected sounds, and processing of sensory information, the only portion relevant to the current discussion is the emission phase. Dolphins can be broken into "whistling" and "non-whistling" species and the ultrasonic emissions of the two types vary (Au 2000). The species that do not whistle include harbor porpoise (*Phocoena phocoena*), finless porpoise (*Neophocaena phocoenoides*), Dall's porpoise (*Phocoenoides dalli*) and Commerson's dolphin (*Cephalorhynchus commersonii*). These species typically have a fairly narrow bandwidth to their echolocation calls with peak frequencies around 120-160 kHz. By contrast whistling species, which include most of the odontocetes, have a much broader bandwidth to their echolocation signals and in fact often have high frequency (> 100 kHz) and lower frequency (30-60 kHz) components. Whistling species can alter which frequency component predominates, with the lower frequency component used in quiet environments and the higher frequency component being used when a large amount of background noise, especially from snapping shrimp, must be overcome (Au 2000). The higher frequency components are also emphasized when a more intense signal is needed and can reach source levels of up to 220 dB. Assuming a source frequency of 100 kHz, a threshold of 170 dB, and an attenuation of 40 dB/km in seawater (Rogers and Cox 1988), an American shad could hypothetically be able to detect a 210 dB dolphin click from 1 km away in seawater (Fig. 1). The actual distance would be expected to be shorter however based on deviations from spherical spreading and absorption by particles in the water. Assuming an absorption of 11 dB/km in seawater (Rogers and Cox 1988) and a threshold of 195 dB, a cod could detect a dolphin echolocation signal of 38 kHz of the same amplitude (210 dB) also up to about 1 km away. Interestingly, dolphins typically have a much quieter signal at lower frequencies and it is not clear that they ever emit lower frequency (30-60 kHz) clicks at a level above the cod threshold of 195 dB (Fig. 1).

If indeed odontocete predation was the selective pressure driving the evolution of ultrasound detection in fish, it seems more likely that fishes evolved ultrasound detection abilities in response to whistling species of cetacean than to non-whistlers. The rather narrow bandwidth of non-whistling species is so far from the normal detection limits of fish that it would be difficult to envision a gradual mechanism for increases in maximum detectable frequency of fish. Hypotheses on the evolution of ultrasound-detecting ability are fraught with difficulties however, as discussed below.

STRUCTURE OF THE GADOID AUDITORY SYSTEM

Because of the relatively high threshold levels to ultrasonic stimuli seen in cod, it has been assumed that the receptors for this stimulation are non-auditory (Astrup and Møhl 1993; Astrup 1999), although there is little evidence for or against this hypothesis as yet. The swimbladder of cod has bilateral extensions which terminate near the base of the cranium (Fig. 2A), very close to the location of the sacculus (Offutt 1974). Ablation of these extensions, as well as ablation of the swimbladder, has profound effects on the thresholds of cod to low frequency (< 300 Hz) stimuli (Offutt 1974) and it is possible that these extensions may play a role in ultrasound detection as well. The resonance frequency of the swimbladder is well below the ultrasonic level (1-2 kHz depending on depth, Sand and Hawkins 1973) but it is possible, although untested, that the extensions may still impart some movements at ultrasonic levels, much as the bullae of clupeoids has a second resonance peak at very high frequencies (Popper et al. 1999). It has been argued that cod are using free nerve endings in the swimbladder to detect vibrations of the swimbladder in response to ultrasound (Astrup 1999). It seems unclear however why this vibration would stimulate these nerve endings without also stimulating the saccular hair cells which lie so close to the swimbladder extensions. Until more in-depth physiological studies are conducted, it remains unclear which sensory system is responsible for ultrasonic detection in cod. If cod can indeed detect and respond to ultrasound, as seems evident from the careful studies on this matter (Astrup and Møhl 1993; Astrup and Møhl 1998), then this should confer an important survival advantage on this species in response to predation by echolocating cetaceans, regardless of which sensory system is being used.

STRUCTURE OF THE CLUPEOID AUDITORY SYSTEM

Fishes in the order Clupeiformes are distinguished by having at least one gas filled bubble, the auditory bulla, in close association with each ear (Nelson 1994). Most species in this order actually have two bubbles associated with each ear, a pro-otic bulla next to the utricle and a more dorsally located pterotic bulla (Fig. 2B). While there is some variation in the structure of these two bullae between species (Blaxter and Hunter 1982), the basic structure is fairly conserved. Clupeoid fish are physostomous (Allen et al. 1976), meaning there is a duct connecting the swimbladder to the stomach, allowing regulation of gas volume in the swimbladder. The posterior half of the swimbladder is thin-walled and compliant but the walls of the anterior half are rigid and surrounded by a thick layer of fibrous connective tissue (Allen et al. 1976). The anterior tip of the swimbladder extends into two fine ducts which enlarge anteriorly into the pro-otic and pterotic bullae (Fig. 2B). This arrangement means the bullae are basically extensions of the swimbladder and gas can be easily exchanged between the swimbladder and bullae, with the swimbladder acting as a gas reservoir for the auditory bullae (Allen et al. 1976).

The structures of the two bullae have important differences which may impact their functional significance. The pterotic bulla is a blind-ended sac that acts as an out pocketing of the duct extending from the swimbladder (Allen et al. 1976). The pterotic bulla is gas-filled in juveniles and adults and typically is situated in the space between the semicircular canals with
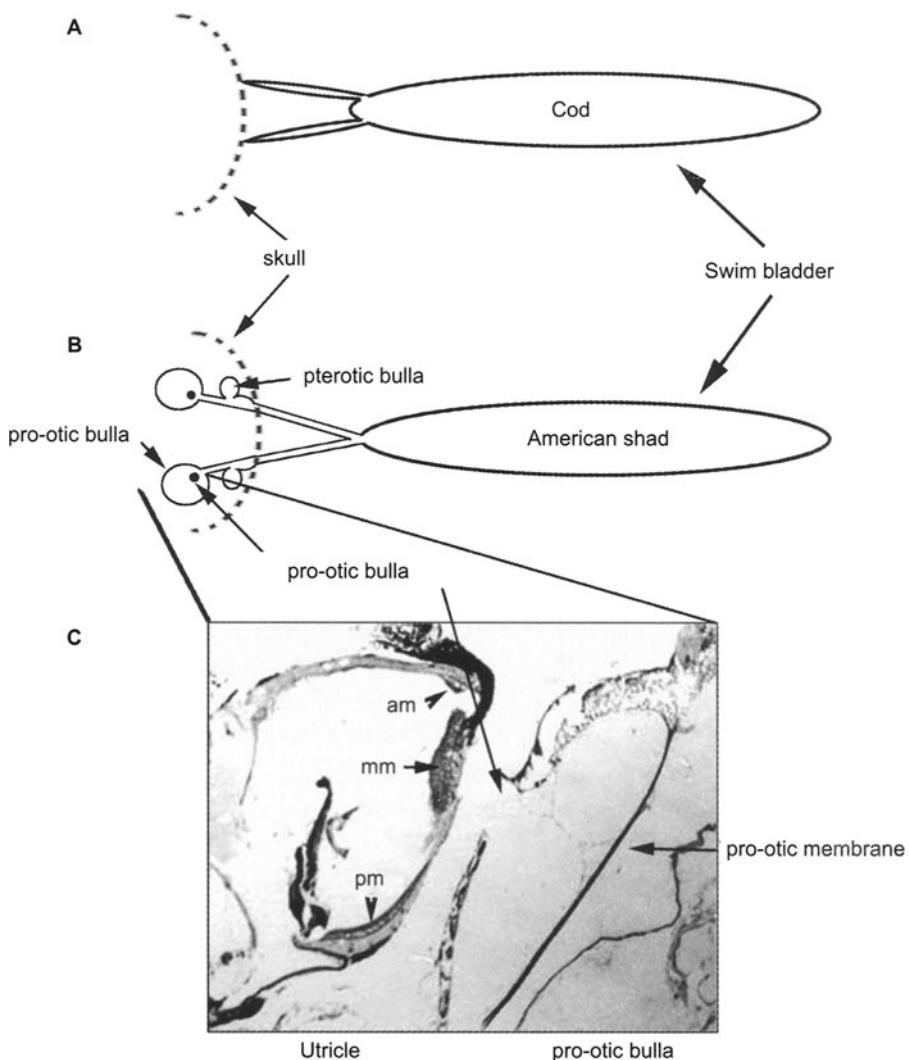
Fig. 2 Diagrammatic representation of a dorsal view of A) the swimbladder and extensions in cod (modified from Offut 1974) and B) of the swimbladder and auditory bullae in a generalized clupeiforme fish (modified from Allen et al. 1976). The pterotic bulla is a simple sac but the pro-otic bulla is divided by a pro-otic membrane into a gas-filled posterior portion and a perilymph-filled anterior portion. C) Section of the pro-otic bulla and utricle of an American shad. The anterior portion of the pro-otic bulla is continuous with the utricle and is connected to the middle utricular macula (mm) by a thin membrane. The middle macula is loosely suspended from the rest of the utricle while the anterior macula (am) and posterior macula (pm) are firmly affixed to the utricular wall. Scale bar = 100 μm.

no direct connection to the ear (O'Connell 1955; Allen et al. 1976). The structure of the pro-otic bulla is more complex. The pro-otic bulla is bisected by an elastic pro-otic membrane which separates the gas-filled posterior portion from the fluid-filled anterior portion (Fig. 2C).

The posterior portion of the pro-otic bulla is continuous with the swimbladder such that changes in swimbladder volume will result in changes in the volume of the posterior portion of the pro-otic bulla. The anterior portion of the pro-otic bulla is continuous with the perilymph of the inner ear through a small opening called the pro-otic fenestra (Fig. 2C). There is also a direct connection between the pro-otic membrane and the utricular epithelium by a thin elastic membrane that is connected to both structures. The pro-otic bulla sits directly beneath the lateral recess membrane (the membrane separating the inner ear and bullae from the cephalic lateral line canals) (O'Connell 1955; Allen et al. 1976).

The utricle of clupeoid fish also shows several specializations that are unique to this group. Unlike all other vertebrates, the utricular epithelium (macula) of fish in this order is separated into three parts (Fig. 2C). The anterior and posterior maculae rest on the wall of the utricle and are firmly attached to the underlying tissue. The middle macula in contrast is suspended from the rest of the utricle and is only connected by a thin layer of connective tissue on each side. It is this middle macula that is connected to the pro-otic membrane via an elastic thread (Best and Gray 1980). The sensory hair cell orientation pattern is also unique in the clupeoid utricle relative to other vertebrates (Popper and Platt 1979). In each utricular macula there are bands of hair cells with opposing polarity, with each direction of maximum sensitivity pointing toward the midline of the macula. The hair cell orientation pattern in the clupeoid utricle looks similar to that seen in saccules and lagenae of other teleosts, both of which are thought to primarily serve an auditory function, suggesting a possible auditory role for the clupeoid utricle. In contrast, in other vertebrates the utricle is thought to be primarily gravistatic and generally has a striolar region curving around the outer edge of the utricle with hair cell orientations pointing toward or away from the periphery (Popper and Platt 1979).

There is also experimental evidence that the structural modifications outlined above are related to hearing ability in clupeoid species. When alternating pressures are applied to a chamber holding anesthetized sprat (*Sprattus sprattus*, Clupeidae) or Atlantic herring (*Clupea harengus*, Clupeidae), the pro-otic bullar membrane moves, also causing movements in the floor of the utricle and in the lateral recess membrane (Denton et al. 1979; Gray and Denton 1979). Both the bullar membrane and the floor of the utricle respond to induced pressure of approximately 50 Hz up to 1000 Hz, with the bullar membrane responding best to frequencies around 500 Hz. Thus the pressure component of sound waves does cause the bullar membrane to vibrate, resulting in movements in the utricular epithelium, acting as an aid to detection of auditory information. This work has now been extended into the ultrasonic range with Popper et al. (1999) showing that the bulla vibrates in response to frequencies of 80-100 kHz. While Popper et al. (1999) were not able to measure movements of the utricle, it seems likely that bullar movements would also be transferred to the utricle at high frequencies.

Of course for movements of the bulla and utricular floor to have any relevance, the sensory receptors in the utricle must be able to respond to auditory stimulation. Due to the fragility of most clupeoid fishes it is difficult to get direct recordings of physiological responses to sound within individual endorgans but there is at least one study that succeeded in this difficult task. Denton and Gray (1979) measured microphonic responses of the utricle of the sprat. Sprat

show utricular microphonic responses to a stimulus frequency range of 5-880 Hz (Denton and Gray 1979). While higher frequencies were not tested, this represents a clear demonstration that the utricle does respond to auditory information.

The final piece of evidence for the importance of the auditory bullae/utricle complex to auditory detection comes from studies of clupeoid behavior in the laboratory. Blaxter and Batty (1985) showed that the behavioral responsiveness of larval herring to a predator increases dramatically after the bulla becomes inflated with gas during development. While an approaching predator will also provide hydrodynamic information, the response seen by Blaxter and Batty (1985) is probably due to acoustic information, since use of an artificial "predator" that supplied mechanosensory (but little auditory) information showed no increase in response upon ontogenetic inflation of the auditory bulla (Higgs and Fuiman 1996). Also, deflation of the auditory bullae in adult herring causes a ten-fold increase in threshold (decrease in sensitivity) to sound stimuli, as measured by startle responses, but ablation of the lateral recess membrane, connecting the pro-otic bulla to the lateral line, has no affect on these thresholds (Blaxter and Hoss 1981). Thus, the auditory bullae do play an important role in detection of auditory stimuli and are probably the predominant structure involved at higher frequencies, although this remains to be definitively proven for ultrasonic frequencies.

EVOLUTION OF ULTRASOUND DETECTION

It has been hypothesized (Astrup and Møhl 1998; Mann et al. 1998) that the main selective force for the evolution of ultrasound detection was predation by echolocating odontocetes. Under this hypothesis, auditory bullae of clupeoid fishes first evolved as a mechanism to increase maximum frequency detection to the higher end of the background spectrum (4-5 kHz or so). This is similar to the hypothesized evolution of the Weberian apparatus of ostariophysans. The ultrasound argument goes further to suggest that the presence of the bulla served as an exaptation, allowing detection up in the ultrasonic ranges as well. There are a couple unanswered questions concerning this hypothesis. The first is that not all species with auditory bullae can detect ultrasound. Using physiological tests, thus far it has been shown that American shad and gulf menhaden show defined responses to ultrasonic tone bursts while bay anchovy, scaled sardine, and Spanish sardine do not (Mann et al. 2001) and yet all five of these species have auditory bullae and a tripartite utricle. If the auditory bullae were the only requirement for ultrasound detection, all clupeiform fishes should be capable of detection of ultrasonic frequencies. That this is not the case suggests there is something special about the auditory system of the Alosinae. Work currently in progress suggests that at least some members of the Alosinae have further specialization in the suspension of the utricular middle macula but more work remains to be done before this is verified.

A second problem with the hypothesis that ultrasonic detection evolved as a response to dolphin predation is why the Alosinae evolved this ability and other clupeoid species did not. There are currently no solid data on the phylogenetics of the Clupeiformes and there is even some doubt on the monophyly of each subfamily within the Clupeidae (Grande 1985). Clupeoid species do not appear to be a heavily favored food item of modern odontocetes (Würtz and

Marrale 1993; Barros and Wells 1998; Walker et al. 1999) and when clupeoids are found in the gut contents of stranded dolphins, ultrasound-detecting and non-ultrasound-detecting species are found in similar proportions (Würtz and Marrale 1993; Barros and Wells 1998). Also, there are no clear habitat distinctions between marine clupeoids that detect ultrasound and those that do not (Table 1) suggesting similar selective pressure. It is possible of course that members of the Alosinae had a utricular suspension more sensitive to higher frequencies before dolphin preda- tion became an issue and therefore selective forces favored retention of this arrangement in descendants. Also, how do cod fit into this evolutionary framework? The phylogenetic distance between gadids and Alosinae clupeids suggests that ultrasound detection evolved at least twice. A more thorough survey of ultrasound detecting ability is needed before the evolution of this trait become clear.

While there are still problems with the current hypothesis that some fishes evolved ultra- sound detection in response to predation by cetaceans, this still seems the most likely explana- tion. There are no other significant sources of ultrasound in the natural environment. Before this hypothesis can be rigorously tested however, we must have much better information on the phylogenetics of the Clupeiformes and a more complete database on clupeoid and gadoid hear- ing abilities must be obtained (see below). Of course we must also guard against the trap that Gould and Lewontin (1979) argued against so forcefully; just because a structure has a given function in current times (e.g. the clupeoid utricle and possible ultrasound detection) does not mean the structure is adapted for that current function. It is possible that the Alosinae just happened to have an ear that detects ultrasound without it being adapted specifically for that function.

PREDATOR AVOIDANCE IN FISH

It has been informally argued that ultrasonic detection abilities in fish would provide little sur- vival advantage because of the relative speed with which a cetacean can swim. Under this argu- ment, by the time a fish has detected an echolocating cetacean, the cetacean has also detected the fish and can catch any fish it may desire. This argument neglects many of the important aspects of successful predator avoidance strategies known for fish. To determine if ultrasonic detection could be a good strategy for avoiding predation by dolphins, a bit more about anti- predator behaviors of fish must be discussed. The predation sequence of any animal has three basic components; encounter, attack, and capture (see review by Fuiman and Magurran 1994). If a prey animal is to successfully avoid a predation event, it must minimize the risk from one or all of these components of predation.

To reduce the probability of encounter, animals must avoid detection by the predator, either through camouflage, seeking refuge, or by avoiding locations containing predators (Fuiman and Magurran 1994). In the context of ultrasound detection by fishes, each of these mechanisms for reducing encounter probability are limited. There is no way for a fish to camouflage itself from ultrasonic detection and, for clupeoids at least, there are few true hiding strategies available since clupeoids are pelagic fishes. If fish can detect the ultrasonic emissions of dolphins, it may be possible for them to avoid an area of active echolocation but this would be difficult. Because of

the higher sensitivity of the dolphin versus fish auditory system to ultrasonic tones (Mann et al. 1998), it is likely a dolphin will detect the echotone from a fish at the same time or before the prey fish detect the dolphin. If a fish of interest were to move away from an area of ultrasonic emissions, it is likely that the cetacean would follow. An avoidance strategy may however be helpful if a fish were coming into an area of already active echolocation, allowing it to avoid areas of high concentrations of dolphin activity. Limiting movement and vertical migration may be one way of reducing encounter probability in clupeoids. While still unquantified, a common behavioral response of ultrasound-detecting clupeoids is to sink low in the water column and to stop most swimming movements, both in the field (Nestler et al. 1992) and in the laboratory (Plachta and Popper, 2003). This may serve to drop the fish out of the sonar beam of the predator and also may reduce the motivation of the dolphin to attack if it senses a non-moving object as non-prey.

To reduce the probability of attack once encountered, animals can increase their apparent size, adopt behaviors designed to confuse the predator, or increase the distance between themselves and the predator (Fuiman and Magurran 1994). Because of differences in swimming speed between dolphins and their fish prey, the last of these three behaviors is not a viable option in the context of ultrasonic detection; both of the other two behaviors should be effective however, particularly in clupeoids. Clupeoid fish are obligate schooling species (Blaxter and Hunter 1982) and schooling can reduce the probability of attack by increasing the apparent size of prey, confusing the attacking predator by movements of the school, or by reducing the individual vulnerability of fish in the school (Pitcher and Parrish 1986). Therefore, upon detection of ultrasonic tones, it would make sense for clupeoid fishes that can detect ultrasound to form tighter schools and this in fact has been reported to happen in laboratory and field studies (Nestler et al. 1992; Plachta and Popper, 2003).

The final way to reduce total vulnerability to predation is to reduce the probability of capture once an attack has commenced. The most effective method of doing this in fish is probably through evasive maneuvers, either by an individual fish (Blaxter and Fuiman 1990; Fuiman 1993) or by an entire school (Breder 1967; Major 1977; Pitcher and Parrish 1986). There have been a number of studies that suggest that escape success is highest for fish that do not respond until a predator is fairly close (e.g. Blaxter and Fuiman 1990; Fuiman 1993; Fuiman and Magurran 1994), although this will to some extent depend upon the species of prey and predator involved. If a prey fish reacts too early to an attack, there is an increased probability that the predator can correct its course and complete the capture. If a prey fish reacts too late to an attack, it may not have sufficient time to avoid capture, especially if the swimming speed of the predator is high relative to that of the prey. In response to ultrasonic tones, American shad show rapid C-start evasive maneuvers (Fig. 3) at stimulus onset. American shad schools break down at high tone intensity levels (above 180 dB) and swimming speed dramatically increases (Plachta and Popper, 2003). Intensity levels above 180 dB probably are experienced by fish in the wild when a dolphin is close, and attacking fish prey. Thus the "panic" responses seen by Plachta and Popper (2003) at high levels may well be a final stage evasive maneuver.
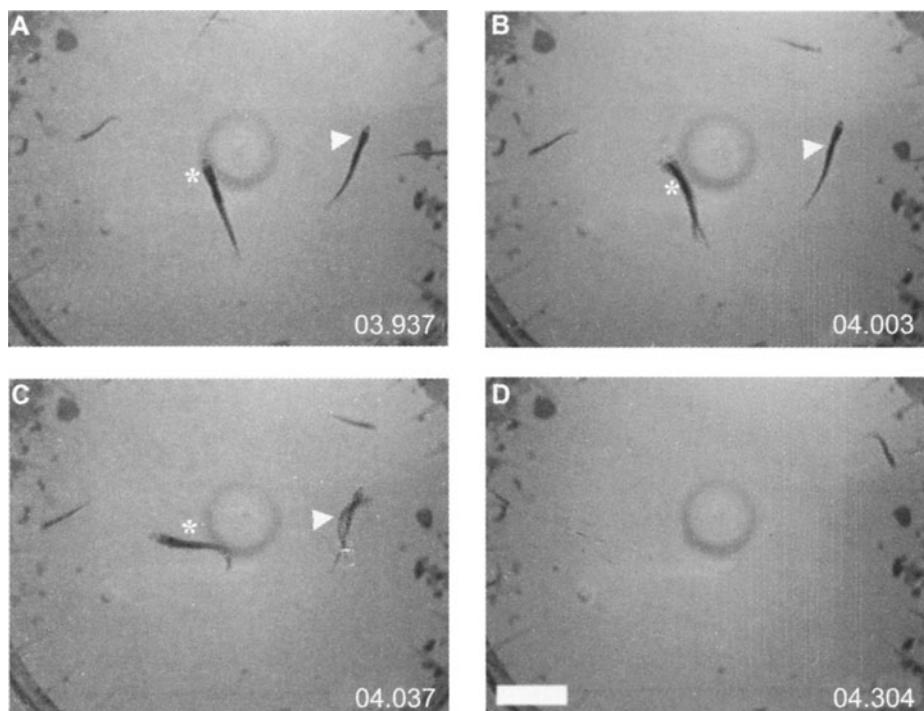
Fig. 3 Example responses of American shad juveniles to a 90 kHz pure tone (intensity = 147 dB). Stimulus onset is at A. Numbers on the bottom of each plate track the time of the response in seconds. At stimulus onset (A) fish are drifting or swimming slowly. Soon after onset (B and C) fish make a rapid C-start acceleration and quickly swim out of the field of view (D). The ultrasonic transducer is just off the bottom right corner of each plate. Two fish are marked with either an asterisk or an arrowhead to facilitate tracking of fish from one frame to the next. Scale bar in D applies to all frames and corresponds to 3 cm.

Based upon all the preceding information, the most effective strategy for fish to reduce their vulnerability to ultrasonic predation may be via late-stage evasive movements during the capture phase of a predatory event. It has been hypothesized, based on the intensity of dolphin ultrasonic emissions and the sensitivity of the ear of ultrasound-detecting species, that these species can detect dolphins from approximately 9-200 m away (Fig. 1, Mann et al. 1998). This is clearly enough time for a fish to prepare evasive movements. It may well be that, instead of relying solely on acoustic signals for final stage evasive maneuvers, fish use auditory information to prepare for a predatory encounter, perhaps by making schools tighter, and that some other sensory modality, vision or mechanoreception perhaps, is used for final stage avoidance. Either way it is clear that detection of ultrasound can confer a selective advantage to prey fish based on all we know about predatory events in fish.

FUTURE EXPERIMENTS

There are many unanswered questions concerning ultrasound detection in fishes, all of which should provide many years of interesting and productive future research.

The most obvious aspect for future investigation is an analysis of just how widespread is ultrasonic detection truly. This ability has only been demonstrated convincingly for one non-clupeoid species and a handful of clupeoid species in one subfamily (the Alosinae). It has been shown to not occur in three other clupeoid fish, leading Mann et al. (2001) to hypothesize that this ability is confined to the Alosinae subfamily within Clupeiformes. A systematic survey of the rest of the order Clupeiformes should be conducted to determine how isolated is the ability to detect ultrasound, although evolutionary implications will be difficult to infer given the confusion still reigning on the status of clupeiform phylogenetics (Grande 1985). This effort could also be expanded to include gadoids and other species that might be expected to have evolved in the presence of dolphin predation as well as those with no cetacean contact to serve as control groups.

To fully determine whether or not ultrasonic detection has any utility as a survival mechanism against dolphin predation, actual trials of dolphin feeding success on the different species must be conducted. While it is difficult to conduct true experiments on dolphins due to the many restrictions, it should be possible to conduct feeding trials with already captive dolphins. This would entail releasing schools of similarly sized ultrasound and non-ultrasound-detecting species into an arena, for example similarly-sized American shad and Spanish sardine, and carefully documenting the capture success of echolocating cetaceans and the behavior of each species. This should provide convincing evidence of whether or not species such as American shad detect ultrasound better than species thought to be insensitive to these high frequencies and whether this detection ability confers any survival advantage.

Another critical question that needs to be answered is: what is the mechanism of ultrasound detection, especially in clupeoids but also in the cod? Single- and multi-unit recordings must be conducted to find neurons in the central nervous system that respond to ultrasonic stimuli. Once these neurons are located, careful labeling and tracing studies should be conducted to determine where these neurons are located and with which other areas of the brain they may communicate. This will be a first step towards determining which sensory modalities these fish are using to detect ultrasound and how these signals might be processed. This approach, or one using more gross-scale measurements such as the auditory brainstem response or conditioning, could then be conducted in conjunction with ablation experiments to knock out one sensory modality at a time. This would help determine what effect the loss of select systems have on ultrasound detection. These ablation experiments could be like those of Blaxter and Hoss (1981), in which structures such as the bulla or lateral recess were ablated, or could represent transection of various auditory or lateral line nerve branches. While these experiments may be difficult, especially on the often fragile clupeoid species, they will be of great utility in determining the sensory modality used for ultrasound detection in fish.

Another interesting avenue for inspection would be the developmental mechanism and timing of ultrasound detection. Clupeoid fishes hatch in a very undeveloped state and do not have a filled pro-otic bulla until well into the larval period (e.g. Allen et al. 1976; Blaxter and Fuiman 1990; Higgs and Fuiman 1996). If the presence of an auditory bulla were a necessary and sufficient condition for detection of ultrasound, fish should be able to hear these high frequencies soon after bulla inflation. Of course many other developmental changes occur to clupeoid sensory systems, including the auditory and mechanoreceptive systems, and an examination of when ultrasound detection develops relative to development of morphological aspects of these systems may provide new information on the sensory modality involved with detection of ultrasound.

Acknowledgements

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The Role of Gas-Holding Structures in Fish Hearing: An Acoustically Evoked Potentials Approach

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ABSTRACT

Fish varies greatly in their sensitivity to underwater acoustic signals. Over the past century, scientists have designed many methods to investigate auditory capacity of fish. Behavioral training paradigms (with either punishment or reward as incentives to the tested subjects) and various electrophysiological methods have been used to obtain audiograms, i.e., the auditory sensitivity curves of fish. Each method has its practical limitations: behavioral methods take long training time while the electrophysiological methods are invasive. This chapter first describes a novel, non-invasive electrophysiological recording method, i.e., the auditory brainstem response (ABR), which was first developed in my laboratory with the purpose of obtaining audiograms within short time for a comprehensive study on fish hearing ability. The setup of the system as well as technical details involved in using the ABR system are discussed in-depth with inputs from various users over the past 5 years since the debut of the ABR method. The second part of this chapter is focused on understanding how gas-holding structures (e.g., gasbladder, suprabranchial chamber, otic gasbladder) play a role in overall hearing ability of fish. The gas-holding structure, e.g., gasbladder, is generally regarded as an underwater resonant bubble. It is assumed that the resonant frequency created by the passing sound wave to gas-holding structure could aid in overall hearing ability of fish. This chapter reviews the work carried out by the ABR method in the author's laboratory investigating how gas-holding structure plays a role in hearing ability of fish. The audiograms are obtained with the use of the ABR method before and after gas removal from: 1) gasbladder of goldfish, toadfish, and blue gourami; 2) suprabranchial chambers of blue gourami, kissing gourami, dwarf gourami; and 3) otic gasbladder of a mormyrid weakly electric fish. The general conclusion is that those fish (e.g., goldfish, gourami, mormyrid) that have direct coupling between gas-holding structure and the inner ear benefits greatly from such a coupling to have better hearing ability. While the gasbladder of toadfish, albeit large in size and is placed in close proximity to the inner ear does not contribute to the overall hearing ability. The finding is in general agreement with how a fish is either classified as a hearing specialist (with coupling device between ear and gas-holding structure) or a hearing generalist (without such a coupling device). This study also reveals the possible existence of different populations of sensory hair cells in specialist species.

Key words: Auditory brainstem response, Electrophysiology, Gasbladder, Audiograms

INTRODUCTION

The perception of underwater sound poses several challenges to fish. Because of similar acoustic impedance between fish body and its surrounding water medium, it makes fish body almost transparent to passing sound waves. Under such condition the fish body vibrates in sync with the sound wave and no differential movement of body is expected to stimulate sensory hair cells. In addition, the high speed of underwater sound (ca. 1500 m/s), long sound wavelengths in water and narrow interaural distance of fish which make the use of temporal and intensity difference cues for localization of a sound source difficult (Bradbury and Vehrencamp 1998). To overcome such physical limitations, chondrichthyians and osteichthyians have evolved to have three otolithic endorgans on each side of head: the utriculus, the lagena and the sacculus, to cope with the constraints. Each endorgan is lined with a tissue matrix in which many sensory hair cells are embedded. As sound passes through a fish and brings its tissue into motion, the otoliths are thought to move at a different phase and amplitude due to their greater mass (e.g., otolith has a density three times that of the surrounding tissues), the stiffness of their attachment to the hair cells and support structures, and their inertia. It is through this mechanism, a relative displacement of the otolith occurs that is in proportion to acoustic particle motion (displacement, velocity, or acceleration), vector quantities, and direction which enables fish to perceive sound (Popper and Fay 1999; Fay and Simmons 1999). Because of the mass of the otolith, such a system can only respond to lower frequencies. Fish solely relies on otolithic organs to perceive sound tend to have the hearing ability that is limited in frequency range and with higher hearing threshold. Fish with limited hearing ability (also known as hearing generalists) are likely to be found in open ocean surf zones, and rushing rivers where noise are abundant at all sound frequencies. Fish in such habitats do not need very sensitive hearing since any soft sounds made by other fish or animals would be masked by the ambient noise.

For those fish live in shallow estuaries and in freshwater lakes and slow streams, the ambient noise levels are much lower. When the water is sufficiently shallow, the reflections of sounds from the surface and the bottom can interact in rather complicated ways. The water then becomes like a large guitar box in which standing waves are set up and there will be some frequencies that build up and propagate well and others that are cancelled out. Under such circumstances, there will be a minimum frequency, i.e., cutoff frequency (F_c), below which no sound can propagate within the layer of water between the surface and the bottom. The value of this cutoff frequency is inversely related to the depth of water. Values of the F_c is also inversely related to the speed of sound in the substrate. For instance, values of F_c range from 400–1100 Hz for water 1 m deep, with higher values for softer bottoms, and 30–200 Hz for water 10 m deep (Bradbury and Vehrencamp 1998). Essentially, the benefit of lower noise in shallow water is partially countered by the heavy attenuation of all frequencies below F_c. The constraints of low noise and the heavy attenuation of those frequencies used by fish both favor more acute auditory sensitivities in shallow water habitats. Many fish in these shallow habitats do evolve to increase sensitivity, localization and frequency range of hearing with a common mechanism. This common mechanism involves with the use of many types of gas-holding structures (e.g., gasbladder in many fish, suprabranchial chamber in anabantoids, otic gasbladder in mormyrids, otic bullae

in herring) to pick up pressure component of a passing sound wave and to couple it to the otolithic endorgans to enhance overall hearing ability of fish. Several folds of benefits can be seen with the use of such a common mechanism. The first advantage is that because the ratio between particle displacement and pressure increases as acoustic impedance of a medium decreases, the lower acoustic impedance of the gasbladder coverts the high pressure-low particle displacements of sound in water into low pressure-high particle displacement in the gas and wall of the bladder. The movement imposed by a bladder on a coupled otolith is thus much greater than the otolith experiences due to movement of nearby water and tissue molecules vibrating with the near field of the sound. Such an arrangement results in an increase in sensitivity in the near field as an end product of the coupling of gas holding devices to the inner ear. The second advantage of this common mechanism is that although gas holding device is primarily a pressure detector and thus it provides no information about sound source angle, however, the knowledge of the pressure amplitude can be combined with the near field information to reduce ambiguities and thus provide directional information. Third, the resonant frequencies of a typical gas bladder are usually in the kilohertz range. The range is significantly higher than that of the otoliths responding to near field force alone. Thus coupling to a gasbladder can extend fish hearing up to 3 kHz. Fourth, a gasbladder coupling allows a fish to respond to acoustic signals in the far field, and thus at greater distances.

For years the role of gas-holding structure (e.g., gasbladder, otic gasbladder, suprabranchial chamber, and otic bullae) in fish hearing enhancement has been studied or speculated. However, due to various methods used in addressing the questions, some conflicting results have led to some arguments as to the essential role of gasbladder plays in the over all hearing enhancement in fish. Recently, a non-invasive electrophysiological recording system, the fish auditory brainstem response (ABR) method (Kenyon et al. 1998) was developed to study auditory physiology of fish. Because of its non-invasive nature, the method is suitable to investigate the role of gas-holding devices in fish hearing enhancement. Hence, an brief description of the ABR method is outlined in the following to give readers an overall idea how it is applied to reveal the role of gas-holding devices in fish hearing enhancement that will be discussed in the later sections.

THE FISH AUDITORY BRAINSTEM RESPONSE (ABR) METHOD

Scientific study on fish hearing dates back to the turn of the 20th century. Parker (1903) was perhaps the first to conduct a well defined experiment investigating the hearing of cyprinid fish and the work was followed by Biglow (1904). Subsequently, quantitative work on the range of frequencies over which fish can hear and on representatives of several different families was carried out by von Frisch and his associates (von Frisch 1936; 1938). Over the years several methods have been developed to investigate fish hearing. Behavioral methods usually involve training fish by using electric shock or food as rewards to respond upon hearing a sound. In the classical conditioning method, fish respond with innate behavior such as stereotyped defense responses (Myrberg and Spires 1980), cardiac suppression (Chapman and Sand 1974) or ventilatory suppression (Banner 1967). The drawbacks of the classical conditioning method include

the stress caused by the electric shock, the response can be ambiguous (especially at threshold level) and is not applicable to species that can not be conditioned by the shock (Nelson 1967). A second behavioral method is the instrumental avoidance conditioning in which fish is trained and learn to cross a barrier in the tank upon hearing a sound to avoid electric shock (Behrend and Bitterman 1962). The advantage of this method is that the response is unambiguous. However, due to free movement of the subject, the precise calibration of sound perceived by fish is difficult to obtain. In addition, the training may require excessive long time (Weiss et al. 1969). A third behavioral method is operant conditioning which involves positive reinforcement of training fish to peck paddles in response to sound (Yan and Popper, 1991, 1992, 1993). The drawbacks of this paradigm are: (1) long duration of training for some species; (2) high degrees of variation in learning among individuals; (3) fish has to be large and responsive enough to perform the paddle packing task. In addition, this method can only be applied to fish using striking mode for food gathering.

On the other hand, electrophysiological methods have less limitation associated with training subjects. Measurement of microphonics from auditory organs while presenting acoustic stimuli to the test subjects is widely used to measure auditory sensitivity of fishes (Saidel and Popper 1987). In addition, single-unit recording is used to measure single nerve fiber discharge patterns (Enger and Anderson 1967). Electrophysiological recordings allow faster data gathering than behavioral methods albeit with some constraints: (1) preparation is complex and invasive surgery is required; (2) the placement of electrodes is restricted to specific endorgans and thus responses recorded do not necessarily represent the whole auditory pathways. A third recording method is the auditory brainstem response (ABR) which is a non-invasive far-field recording of synchronous neural activity in the eighth nerve and brainstem auditory nuclei elicited by acoustic stimuli (Jewett 1970).

The purpose of this chapter is to summarize the ABR recording method (Kenyon et al. 1998) developed in my laboratory and to share with colleagues the results of the past 6 years on the usage of this protocol in the study of the role of gas-holding structures in fish hearing.

THE FISH ABR SET-UP

The block diagram in Figure 1 shows the overall layout of the ABR system. The system uses a Windows-based PC to control 7 electronic modules for signal conditioning which are manufactured by the Tucker Davis Technologies (Gainsville, Florida). Sound stimuli waveforms are generated with TDT "Sig-Gen" software and short (20 ms) pure tone bursts are presented with "Bio-Sig" software through a DA1 (digital-analog converter) and a PA4 (programmable attenuator) to a power amplifier via speaker to broadcast stimuli to the subjects. A microphone is placed inside the soundproof chamber and in conjunction with a MA1 (microphone amplifier) and a MC1 (monitor-speaker) to monitor the presentation of acoustic signals inside the chamber.

Test subjects are sedated with the injection of gallamine triethioide (G-1137, Sigma Chemicals Co., St. Louis, Missouri, USA) and secured with the aid of a micromanipulator inside a rectangular 15-liter plastic tub filled with water. A reservoir placed inside the sound
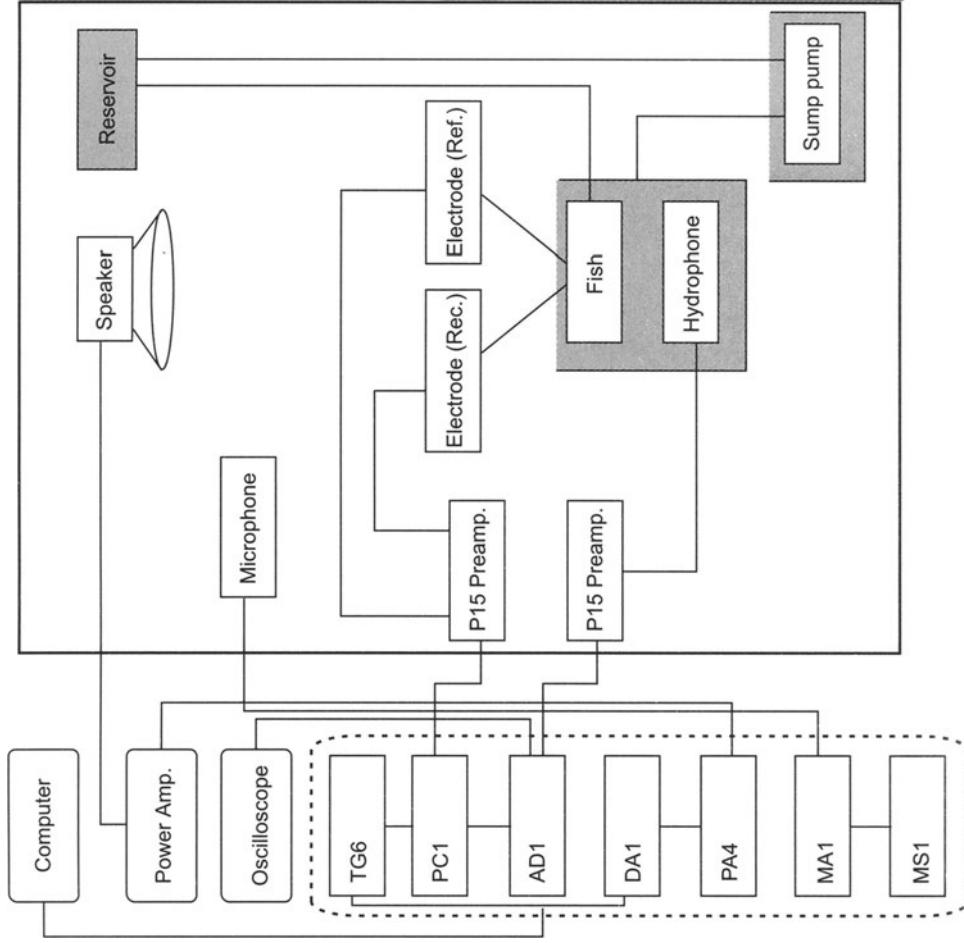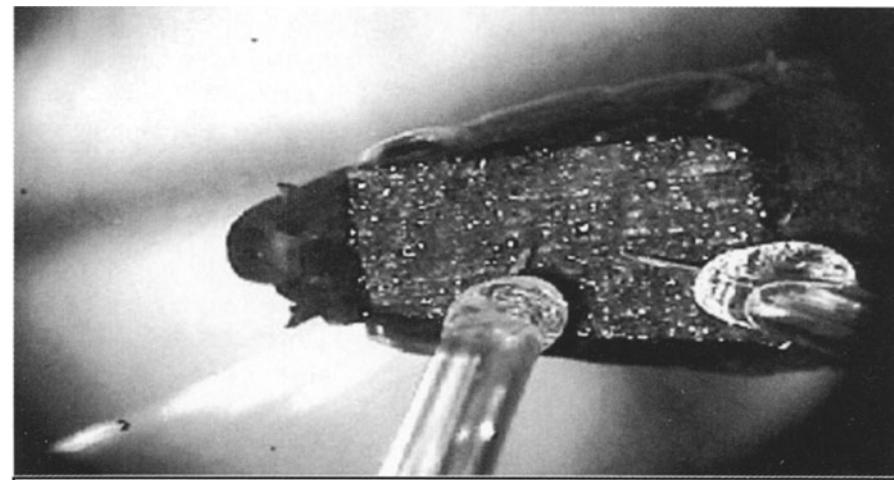
Fig. 1 Block diagram of the fish auditory brainstem response (ABR) recording system. Components inside the soundproof chamber are enclosed by bold lines while signal conditioning modules are enclosed by dashed lines. Photo image on the right shows two electrodes positioning on the fish head as viewed under a stereo dissecting microscope.

proof chamber provides gravity-feed highly oxygenated water to keep fish alive during the recording. The overflowed water is siphoned out to a tank that holds a sump pump to recycle water back to the reservoir. The height of the fish is adjusted so that the nape region is about 0.5-1.0 mm above the water. One Teflon-coated silver recording electrode is placed on the midline of the skull over the medulla region while the reference electrode is placed about 5 mm anterior to the recording electrode. A hydrophone is placed inside the tub right next to the head region of the subject in order to calibrate the sound pressure level presumably perceived by the fish. The acoustically evoked potentials are recorded by electrodes and are amplified 100× by a first stage Grass P-15 amplifier and are fed into a spike conditioner (PC1) and amplified 100× again. The recorded brainwaves are routed into the computer and are averaged by "Bio-Sig" software. A TG6 (timing generator) is used to synchronize A/D and D/A conversion. The oscilloscope is used to monitor real time brainwaves and output of hydrophone.

The recorded ABR traces of opposing polarities (one thousand sweeps each) are averaged together forming a 2000-sweep trace to eliminate any stimulus artifact. At each test frequency, this is done twice and is overlaid to examine if traces are repeatable. The lowest sound pressure level where a repeatable ABR can be obtained, as determined by overlaying replicate traces, is considered the threshold. In addition to the visual inspection method, a Spearman Rank Order correlation coefficient with r value less than 0.3 between two traces also indicates that two traces are not repeatable and is hence considered below the threshold.

GASBLADDER, SUPRABRANCHIAL CHAMBER AND OTIC GASBLADDER

Goldfish use Weberian ossicles to mechanically couple gasbladder to the inner ear. Gouramis holds air inside the suprabranchial chamber which is in close proximity to the inner ear. Mormyrid weakly electric fish has an otic gasbladder tightly coupled to the saccule on each side of inner ear. These tightly coupled gas-holding structures have been suggested to pick up pressure component of the passing sound waves and transmit it into the inner ear to enhance overall hearing. To test this hypothesis, goldfish (*Carassius auratus*), blue gourami (*Trichogaster trichopterus*), kissing gourami (*Helostoma temminckii*), dwarf gourami (*Colisa lalia*), and a mormyrid (*Brienomyrus brachyistius*), all have coupled gas-holding structures are used. In addition, the oyster toadfish (*Opsanus tau*) which does not have any coupling between the gasbladder and the inner ear but with close proximity to each other is used as a control to demonstrate the effectiveness of mechanical coupling in hearing enhancement. Radiographs are taken for each species to help localizing the position of gas-holding structures. The baseline audiograms are taken with the ABR protocol. The gas inside the gas-holding structures is removed either by needle attached to a syringe (for gasbladder and otic gasbladder) or flush out with water (for suprabranchial chamber) with the aid of a micropipette tip. The audiograms are taken again after removal of gas.

A series of typical acoustically evoked brainwaves are illustrated in Figure 2. It consists of a series of pronounced peaks. When the stimuli is attenuated, the peak to peak amplitudes of the evoked potentials are also reduced accordingly. The obvious sign of brainwave generated below threshold is when two traces can not replicate itself and in many places opposite polarities are observed (see Fig. 2, 60 dB traces).

The audiogram of goldfish clearly indicates that at least it can hear up to 4 kHz and with best hearing frequency between 500 and 800 Hz (Figure 3; lower trace). Upon the removal of gas from the gasbladder, significant increase in thresholds is observed in all frequencies (Figure 3, upper trace). The removal of gas de-couples the mechanical link between the gasbladder and the inner ear. The removal of gas from suprabranchial chamber also caused
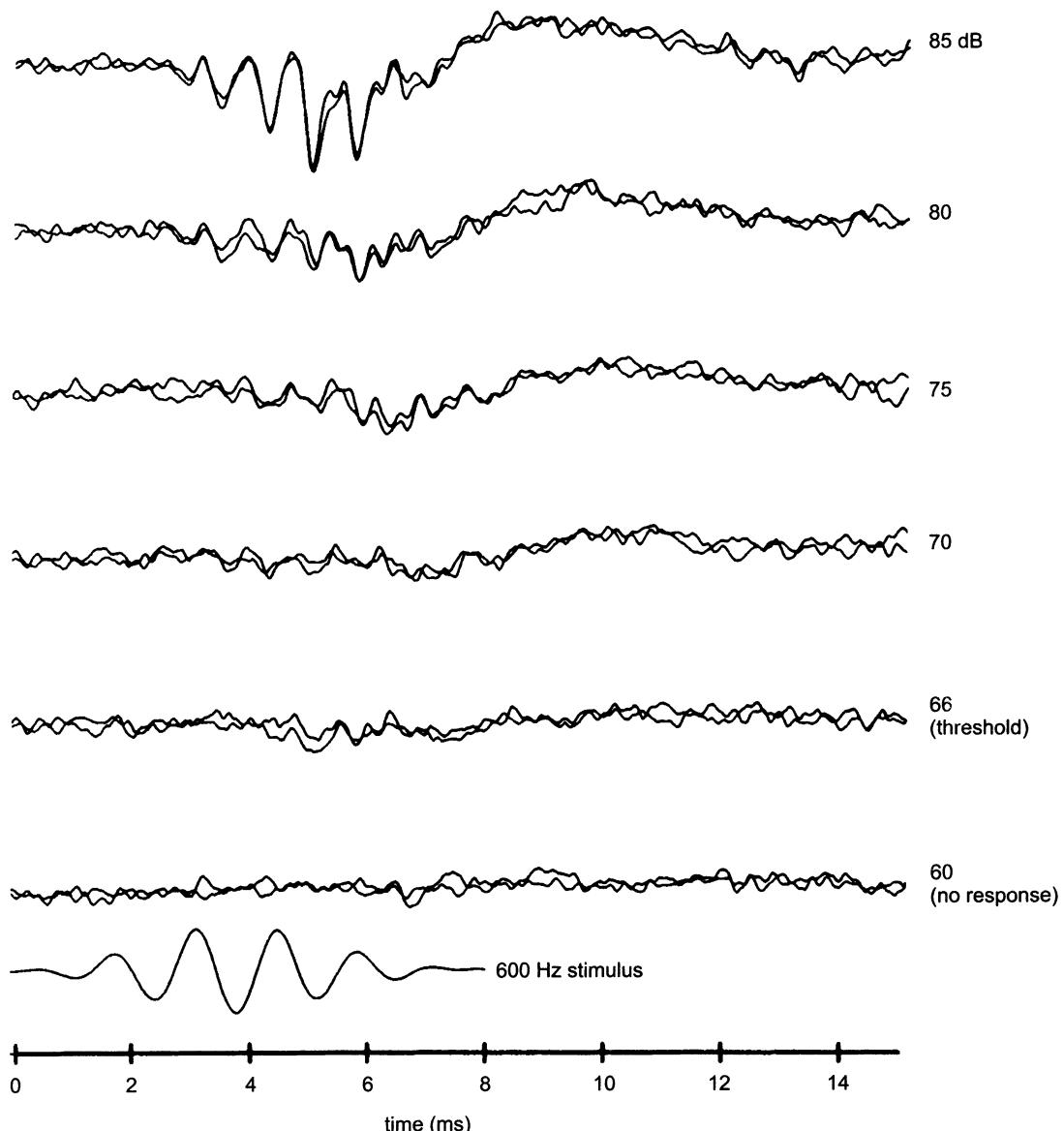
Fig. 2 Acoustically evoked brainwaves of a goldfish in response to a 600-Hz tone burst attenuated in 5-dB steps. Averaged traces of two different runs for each sound pressure level are overlaid. dB here is in relative scale. (Data from Kenyon et al. 1998).

significant elevation of thresholds in blue gourami (*Trichogaster trichopterus*), kissing gourami (*Helostoma temminckii*) and dwarf gourami (*Colisa lalia*) (Figure 4, 5, 6)(see Yan 1998 for details).

The presence of otic gasbladder on each side of ear provides an unique chance to examine how individual otic gasbladder plays a role in modulating the overall hearing ability of a mormyrid weakly electric fish (*Brienomyrus brachyistius*).

The results in Figure 7 indicate that significant increase of hearing thresholds are observed after both sides of otic gasbladder are deflated. However, removal of gas from just one side of otic gasbladder does not result in any significant change of threshold when compares to the baseline data. The acoustically evoked brainwaves in Figure 8 also illustrate the reduction of potentials after both otic gasbladders are deflated (Trace C). Only moderate reduction of potentials is seen in fish with one side of otic gasbladder deflated (see Yan and Curtsinger 2000 for details).
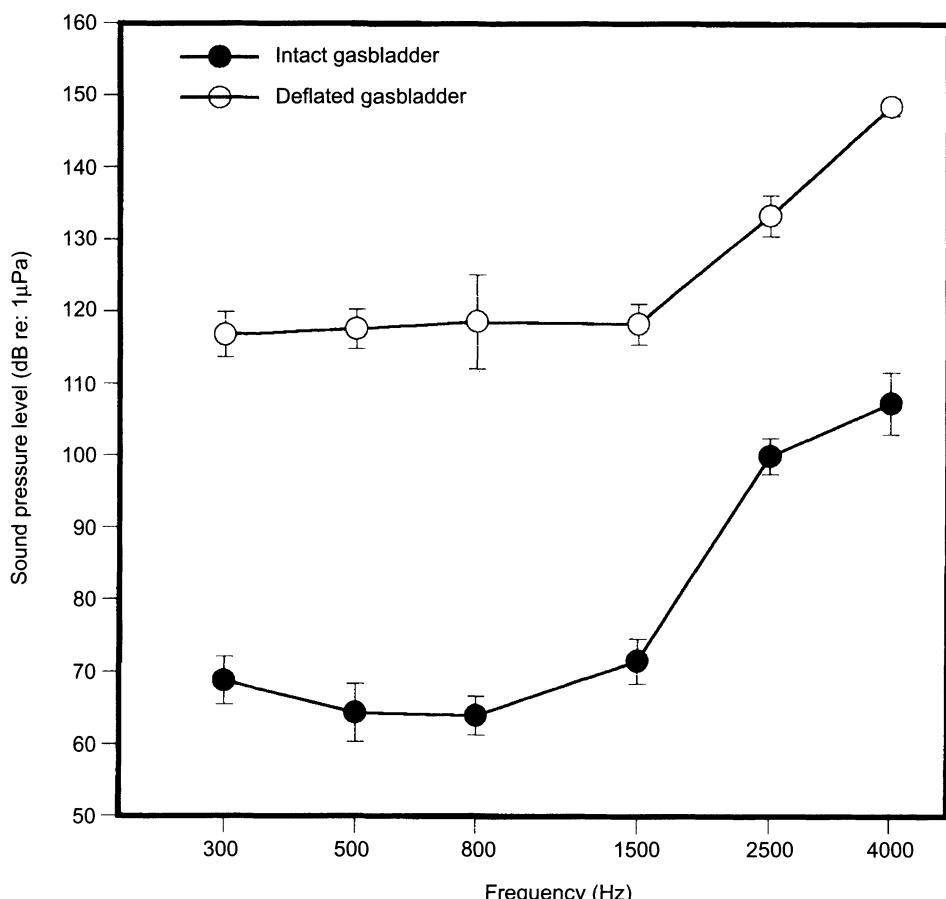
Fig. 3 Audiograms of goldfish with intact gasbladder (solid circles) and deflated (open circles). Error bars indicate standard error. N = 6. (Data from Yan et al. 2000).
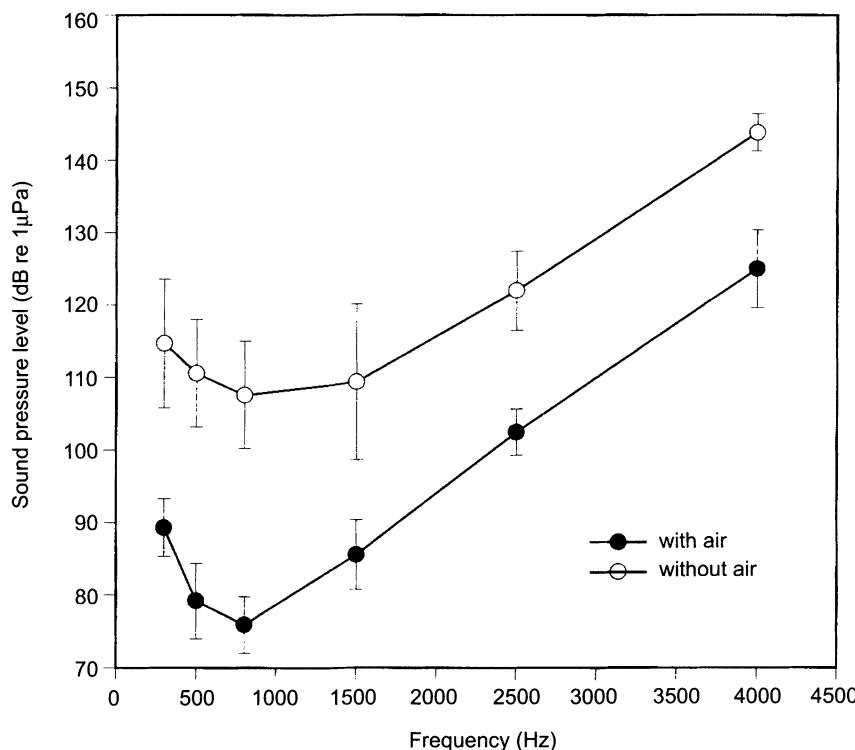
Fig. 4 The audiograms of blue gourami before (solid circles) and after (open circles) removal of air bubbles from the suprabranchial chambers. Error bars indicate standard error. N = 5. (Data from Yan 1998).

The oyster toadfish is the only species examined that does not have any mechanical coupling between gas holding structure (e.g., gasbladder) and the inner ear. In terms of relative distance between the gas holding device and the inner ear, the toadfish has the closest distance between two structures among 6 species examined. Nevertheless, the removal of gas from gasbladder of toadfish does not result in any change of hearing threshold. Based on the results obtained from 6 species examined, it is concluded that mechanical coupling between gas-holding device and inner does play a major role in enhancing overall hearing ability of certain groups of fish.

The acoustico-lateralis system of the sprat (*Clupea sprattus*) and other clupeids has two partly gas-filled bony bullae which transform pressure changes into liquid displacements capable of stimulating the sense organ of the ear and lateral line (Allen et al. 1976, Gray and Denton 1979, Denton et al. 1979). It is found that the utricle is a very sensitive sound pressure detector which has one population of receptors that respond to the decompression of a sound wave (Denton and Gray 1979). Recently, the American shad (*Alosa sapidissima*), a member of order Clupeiforms, is found to have perception of ultrasound (up to 180 kHz) by the use of classical conditioning method (Mann et al. 1997; Higgs, this volume). With the use of fish ABR system,
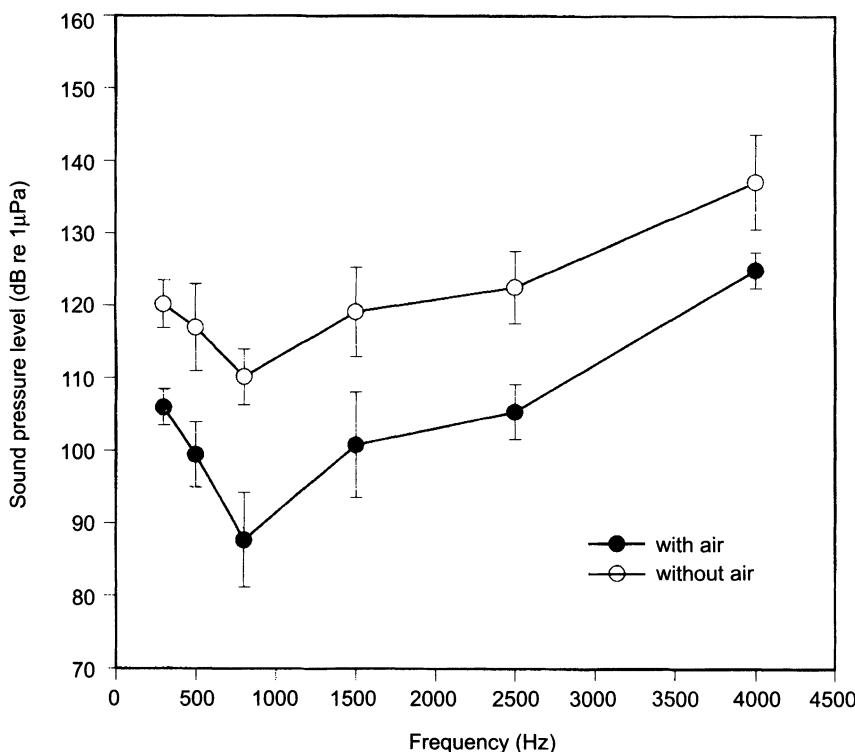
Fig. 5   The audiograms of kissing gourami before (solid circles) and after (open circles) removal of air bubbles from the suprabranchial chambers. Error bars indicate standard error. N = 5. (Data from Yan 1998).

Mann et al. (2001) found an additional species, the Gulf menhaden (*Brevoortia patronus*) is able to detect ultrasound while several other species including the bay anchovy (*Anchoa mitchilli*), scaled sardine (*Harengula jaguana*), and Spanish sardine (*Sardinella aurita*) only detect sounds to about 4 kHz. It is known that among Clupeiforms the swimbladder is tightly connected to pro-otic and pterotic bullae which connect directly to inner ear (Allen et al. 1979), therefore, to what extent the gasbladder of Clupeiform fish contribute to the overall hearing, especially for those species that could hear to ultrasound range, remains to be addressed.

DISCUSSION

The conventional psychophysical methods (e.g., heart beat rate conditioning; underwater paddle pecking) of measuring audiograms from fish can take up to weeks or even months to complete (Yan and Popper 1991, Yan 1995). The ABR recording method offers a rather rapid acquisition (i.e., within days) of data which enables researchers to conduct a broader scale of comparative study on fish hearing. The non-invasive recording approach also has the advantage of repeated recordings from the same subject after receiving experimental treatment, e.g., withdrawal of gas from gas-holding structure (Yan 1995, 1998, Yan et al. 2000) or noise exposure
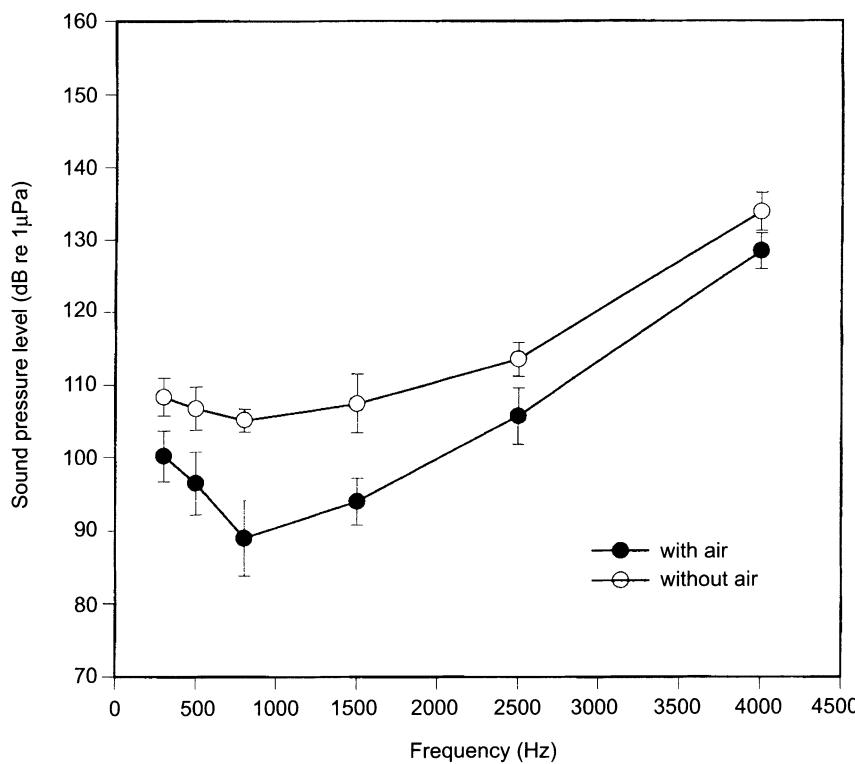
Fig. 6 The audiograms of dwarf gourami before (solid circles) and after (open circles) removal of air bubbles from the suprabranchial chambers. Error bars indicate standard error. N = 5. (Data from Yan 1998).

(Scholik and Yan 2001, 2002a, b). The smallest size of larval fish recorded so far is 8 mm (Simpson et al. 2002, Yan et al. 2002) and therefore the ABR technique offers a practical way of investigating ontogenetic changes of hearing ability of fish (also see Wysocki and Ladich 2001, Higgs et al. 2002, Yan et al. 2002, Simpson et al. 2002, Higgs, this volume; Ladich, this volume) which is impossible if the traditional psychophysical methods or other electrophysiological methods were to be used. The additional advantage of the ABR method is that it records overall responses to acoustic stimuli along the ascending auditory neuronal pathways (Hall 1992) which certainly gives better resolution than those obtained by microphonics or single unit recordings. It is important to point out, however, that the ABR technique does have its limitations. Since it records the overall acoustically evoked responses from many neuronal generators along the auditory pathways, how each generator contributes to the overall hearing is not clear. This discrepancy of understanding has to wait until detailed mapping of auditory pathways of each fish species as well as recording from experimental lesions to specific neuro-generators are achieved. In addition, it has been well documented that audiograms generated from electro-physiological recordings tend to have higher thresholds than those obtained by behavioral methods (Gorga et al. 1988; Kenyon et al. 1998). Therefore, it is suggested that the same acous-
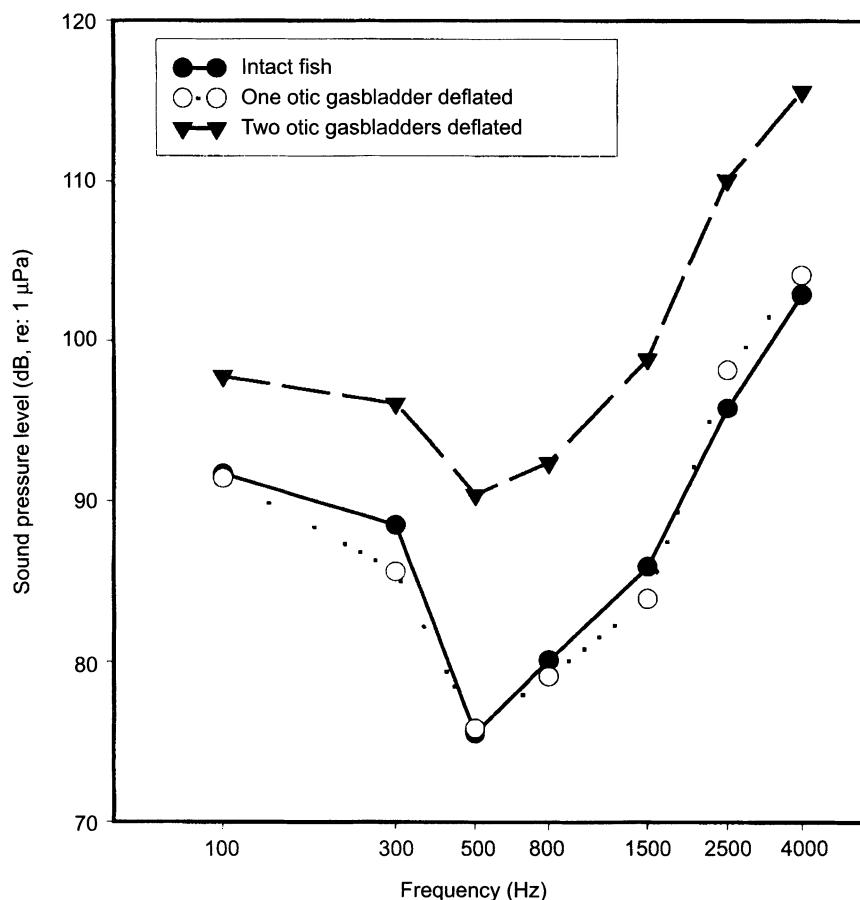
Fig. 7 The audiograms of a mormyrid weakly electric fish (*Brienomyrus brachyistius*) obtained from three experimental conditions. Solid circles: otic gasbladder intact fish (N = 4). Open circles: one side of otic gasbladder deflated (N = 4). Solid triangles: two sides of otic gasbladders deflated (N = 4). (Data from Yan and Curtsinger 2000).

tic presentation system used to acquire ABR audiograms should be modified to obtain behavioral audiograms in order to compare possible existence of differences of two types of audiograms obtained.

Feedback from many colleagues who adopted the ABR system that was developed in my laboratory (Kenyon et al. 1998) indicates that the vibration of recording electrodes in response to low frequency (less than 200 Hz) acoustic stimuli tends to cause artifacts which are incorporated into averaged responses. Our original report (Kenyon et al. 1998) does not offer detailed description on the fabrication of the electrode. The following figure (Figure 10) gives a detailed description of the electrode.

The key point of electrode fabrication is to provide rigidity to the electrode. The first step is to encase Teflon–coated silver wire with capillary glass tube. It is then housed inside a 10-cm
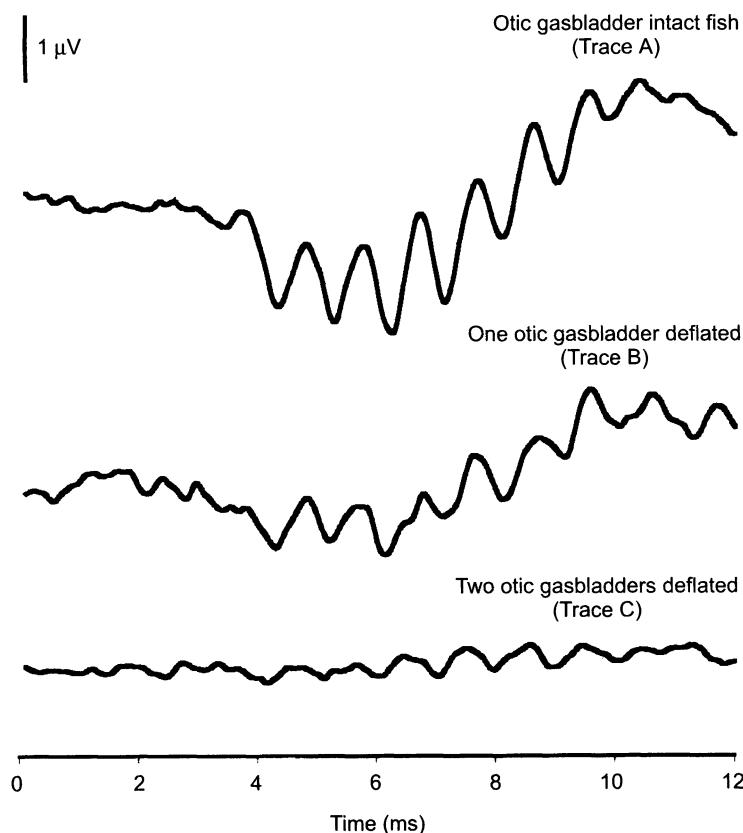
Fig. 8 The acoustically evoked brainwaves of *Brienomyrus brachyistius* in response to 500-Hz, 125-dB tone
burst. Trace A: otic gasbladder intact fish; Trace B: one side of otic gasbladder deflated; Trace C: two
sides of otic gasbladder deflated. (Data from Yan and Curtsinger 2000)

long plastic pipette. A glass pipette can also be used to substitute the plastic one. However, plastic pipette is easier to saw off with appropriate length as required by the setup. Both ends of the electrode are sealed with Epoxy glue to the pipette to provide firm contact with the housing unit and to prevent any in sync movement with the acoustic signals. The whole electrode is clamped firmly to a micromanipulator. During the recording, the tip of electrodes have to be pushed firmly against the skin of the skull of fish to avoid any in sync vibration with acoustic signals, in particular at low frequency range. A simple way to detect whether vibrational artifacts are picked up through the electrodes is to run a test run of ABR recording with a preserved fish under highest sound pressure that can be generated from the system. A dead fish should not generate any physiological evoked potentials and the averaged response should show only a flattened wavy line representing noises from the system. When two replicated from preserved fish are overlaid both traces should not be identical due to random noise process. In addition, 2 or 3 attenuation levels of ABR should also be generated and no difference of these waves should be noticed because dead fish should have no response to different sound pressure levels.
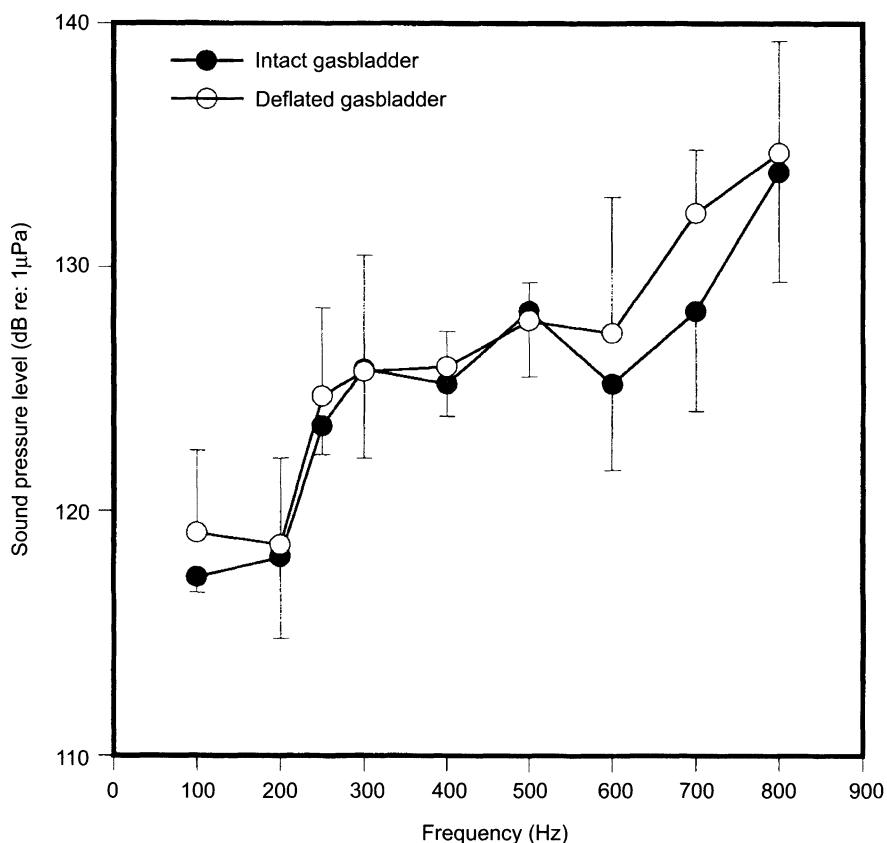
Fig. 9 The audiograms of oyster toadfish before (solid circles) and after (open circles) removal of air bubbles. Error bars indicate standard error. N = 5. (Data from Yan et al. 2000).
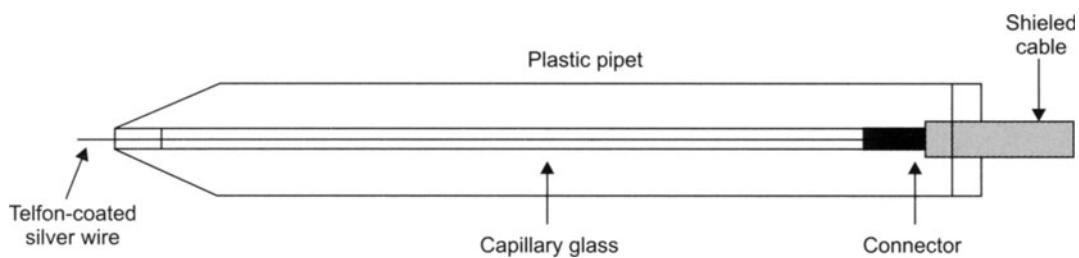
Fig. 10 A schematic diagram of the configuration of recording and reference electrodes used in fish ABR recording.

Myogenic noises can distort averaged brainwaves. To avoid this artifact some form of neuromuscular junction (NMJ) blocker has to be administered to the fish prior to the tests. A very widely used chemical is gallamine triethiodide, an antagonist for muscarinic acetylcholine receptors. The dosage, however, has to be empirically determined due to great latitude of

responses to this chemical in different species. Among some 30+ species of fish examined so far in my laboratory, only one species, the Little skate (*Leucoraja erinacea*) fails to respond to this neuromuscular junction blocker. However, d-tubocurarine chloride (T2379, Sigma Chemicals) is found to be able to sedate the animals (Casper et al., in press). Gallamine trithiodide exerts its blocking effect on all teleosts tested but fails on Little skate, the only elasmobranch examined. This observation indicates that there are possible different types of neurotransmitters or receptors to the neurotransmitters between elasmobranchs and teleosts. Further experiments are warranted to delineate the differential responses between two groups of fish to gallamine triethiodide.

Tight coupling of the posterior element of the Weberian ossicles to the gasbladder in goldfish is very obvious under the view of radiograph (Yan et al. 2000). The removal of gas from the gasbladder essentially renders the function of Weberian ossicles obsolete. The significant elevation of hearing thresholds (Figure 3) after gas removal supports the long-held hypothesis (Frisch 1936, 1938) that mechanically coupled gasbladder into inner ear does assist in fish hearing. This change is also evident by a simple examination of the changes of amplitude of evoked brainwaves before and after gas removal. For example, a goldfish shows a peak-to-peak potential of about 2.35 μV in response to a signal of 300 Hz tone burst at 132 dB. After the deflation, the amplitude drops to 0.3 μV with the same stimulus (Yan et al. 2000). The elevated thresholds return to its baseline values 7 days later after deflated gasbladders are allowed to refill (Yan et al. 2000). These data further validate the role of coupling between inner ear and gasbladder in enhancing overall hearing of goldfish (Figure 3).

Most of fish houses their inner ear inside the skull to protect this vital sensory organ. The layout of inner ears in gouramis is an exception to the norm. The otic capsule that contains the three hearing endorgans is encased in a membrane-like thin bony structure and protrudes into the suprabranchial chamber. The advantage of such an anatomical arrangement can be interpreted as to maximize the exposure of inner ear to the compressible gas held inside the chamber to enhance their hearing. Significant elevation of thresholds to three gouramis examined after removal of gas from the suprabranchial chamber. In addition, refilling of gas inside the chamber brings thresholds back to baseline level support the hypothesis that gas held inside the chamber facilitates and enhances overall hearing of gouramis (Figures 4, 5, 6)(Yan 1998).

The direct coupling of otic gasbladder to the saccule of mormyrids prompts von Frisch to suggest it may play a role in hearing enhancement (Frisch 1938). The deflation of both sides of otic gasbladder leads to significant elevation of hearing threshold (Figure 7) as well as reduction of evoked potentials (Figure 8). Recently, a behavioral method is used to investigate the role of otic gasbladder on three sound-producing mormyrid fish (Fletcher and Crawford 2001). They found that the tympanic bladders increase auditory sensitivity by approximately 30 dB in the middle of the animal's hearing range. Thus, in conjunction with our earlier finding (Yan and Curtsinger 2000) the hypothesis first raised by von Frisch that the tightly coupled otic gasbladders can transmit the sound pressure component of the acoustic signals into the inner ear to enhance overall hearing ability in mormyrids is further confirmed. However, the finding that deflation of only side of otic gasbladder does not result in any significant change of hearing

threshold (Yan and Curtsinger 2000, Fletcher and Crawford 2001) is somewhat intriguing. Our hypothesis originally predicted that deflation of only one side of otic gasbladder should lead to an elevated threshold in between those of baseline and deflation of both otic gasbladders. Interestingly clinical work has demonstrated monaural stimulation of human subjects resulted in an acoustically evoked brainwave that is similar in shape but with reduced amplitude when compared to bilaterally-stimulated (Levine 1981). A similar finding is observed in mormyrid work (Yan and Curtsinger 2000) when comparing trace B (one otic gasbladder deflated) with trace A (otic gasbladder intact fish) of Figure 8. Even with deflation of one otic gasbladder, the saccule should be still functional, albeit with less sensitivity. The acoustically evoked brainwave is the summation of neuronal activities from various neurogenerators at different sites in the ascending auditory pathways (Hall 1992). In humans, the first bilateral representation of acoustic stimuli occurs at the olivary complex where it receives inputs from both ipsilateral and contralateral cochlear nuclei (Hall 1992, Yost 1994). Therefore, as long as one ear is properly stimulated, neurogenerators on both ipsilateral and contralateral sides of the ascending pathways above the first cross over site (the olivary complex) are stimulated. Hence, the overall acoustically evoked waveform does not deviate greatly from the waveform generated in monaurally and binaurally stimulated subjects (Levine 1981). The auditory pathway of a mormyrid (Pollimyrus isidori) has been well mapped (Kozloski and Crawford 1998). The bilateral projection pattern of saccular nerve fibers into dorsomedial zone of the descending octaval nucleus (dzD) suggests that auditory inputs from each ear may be combined early in auditory processing at this major first-order nucleus. It is suggested that the bilateral projection pattern of primary afferents into dzD may have evolved to integrate information from the two ears and to create a fused representation of the pressure component of the sound field (Kozloski and Crawford 1998). Assuming a similar pathways in B. brachyistius then it is possible that B. brachyistius integrates acoustic inputs from both ipsi- and contralateral sides of ears at the dzD nucleus. This may explain why there is little difference in the acoustically evoked brainwave between otic gasbladder-intact and one-side deflated fish (Figure 8; trace A vs. trace B). Unilateral deflation of otic gasbladder in three mormyrids also yield similar findings (Flectcher and Crawford 2001) like that of Yan and Curtsinger (2000) further confirms this argument.

There is an unexpected finding in the organization of otic gasbladder in B. brachyistius. Histological sections reveal a tight coupling between the otic gasbladder and the saccule as was first suggested by von Frisch (1938) and Stipetic (1939). However, about 20% in distance from the anterior tip of the otic gasbladder, a thin and slightly slanted septum (about 2 μm in thickness) separates the otic gasbladder into two unequal chambers (see Figure 2 in Yan and Curtsinger 2000). In light of this finding an interesting question to ask is what kind of function of acoustic sensation is served by such a two-chamber amplification design? This intriguing question requires further study.

Gasbladders have been compared to pulsating underwater bubbles that are strongly resonant structures (Harris 1964). The gas inside the gasbladder is readily compressible and pulsates when exposed to sound, re-radiating the sound in all directions, including toward the ears (McCartney and Stubbs 1971). van Bergijk (1967) even argues that "a fish with a swim

bladder is thus potentially sensitive to a spectrum of far-field sounds ranging in frequency from below 100 Hz to somewhere in the low kHz range since the swim bladder is a simple resonating device." The notion that gasbladder aids in underwater hearing is widely accepted and can be seen in many fish biology and ichthyology textbooks (Moyle and Cech 1996; Helfman et al. 1997). The deflation of gasbladder of oyster toadfish shows no significant change of thresholds (Yan et al. 2000) indicating that the accepted notion may not be completely correct. However, Hastings (2002, personal communication) suggests that since the ABR recording is made near the water surface, the water pressure (due to various depth) may not be taken into account after gas deflation in oyster toadfish which may explain no contribution of gasbladder to its overall hearing. Hastings cautions that further tests on holding fish in deeper depth may yield different results and this line of work remains to be done to sort out the possible confusion as suggested by the present data. However, in a study Sand and Hawkins (1973) measured resonance frequency and damping of the swimbladder of living cod (a hearing generalist with no connection between swimbladder and inner ear) at different depths. They found that at adaptation depth, the resonance frequency (f_r) of the organ was much higher than that predicted for an unrestrained gas bubble of similar volume. However, at much greater depths (where the hydrostatic pressure was 2 or more times greater than the adaptation pressure) f_r was only higher than expected by a factor of 1.25, and changed with depth in the manner of a free gas bubble. Thus, they concluded that the maintenance of an f_r well above the hearing range of the fish, ensures that the relative sensitivity of the animal to different frequencies does not alter with changes in depth. However, taken together the findings from goldfish, gouramis, mormyrid and compare it with the finding in oyster toadfish, it is clear that only gas-holding structure that has direct or mechanical coupling between gas-holding device and the inner ear can enhance hearing in fish.

It has been widely accepted that species having a particularly efficient mechanical coupling between the gas-filled chamber and the otolith organs, i.e., the hearing specialists, tend to have high sensitivity to sound pressure and may hear in a relatively wide frequency range (Popper and Fay 1993, 1997, 1999). The results of gas-holding device deflation experiments clearly demonstrate elevation of hearing thresholds after the treatment (Figures 3-7). However, removal of gas fails to reduce any change of hearing frequency range in hearing specialists and it seems to contradict the long held belief of the role of coupled gas-holding structure in extending the hearing frequency range. The observed fact points to a possibility that distinct differences exist between the sensory hair cells of hearing generalists and hearing specialists, i.e., population of hair cells coded for higher frequency hearing exist only in hearing specialists. Further experiments are needed to elucidate this issue.

Fish hearing research, at least in terms of measurement of audiograms, has long lagged behind those of mammalian and avian species study despite more than half of the numbers of vertebrate species are fish. The constraint of physical media, i.e., water, is a major limiting factor and the lack of a rapid protocol of measurement is another technical bottleneck. The auditory brainstem response protocol reported and discussed here represents an improvement in the pace of acquiring baseline hearing ability data of fishes. The noninvasive nature of the technique also provides researcher greater latitude of manipulation in the treatments of subjects for

various experimental purposes to address more questions relate to fundamental hearing ability of fishes. Since its publication, the fish ABR method has gained worldwide use in the study of animal hearing including fish, amphibian, reptile and bird and more than 20 papers have been published so far using the fish ABR method. The method has already shown its potential in accelerating the comparative study on fish hearing.

The removal of gas from gas-holding structures confirms its role in the enhancement of hearing, at least in terms of hearing threshold. However, it is also discovered that such a device in fact does not necessary contribute to the enhancement of hearing frequency. It remains an enigma as to how wider frequency range is achieved among hearing specialist species.

Acknowledgments

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10

Sound Production and Acoustic Communication

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ABSTRACT

Fishes have evolved a diversity of sound-generating organs. These include vibrating the swimbladder and pectoral girdle by rapidly contracting muscles or rubbing bony elements against each other (stridulation) and plucking enhanced tendons. While the former mechanisms produce low-frequency, often harmonic signals (< 500 Hz), the latter usually generate broad-band pulsed sounds with frequencies up to a few kHz. The restriction of fish sounds to lower frequencies limits the distances over which sounds can propagate, especially in shallow waters where sound transmission is negligible below a certain frequency (cut-off frequency).

Sounds are uttered in a variety of behavioral contexts, especially during agonistic interactions, courtship, spawning and in distress situations such as when they are disturbed or caught. The functional significance of sounds has seldom been investigated despite a wealth of behavioral studies. Acoustic signals may serve in reducing aggression, in assessing of the fighting ability of opponents, in species recognition, in attraction of mates and in mate choice.

Is acoustic communication a driving force in the evolution of hearing sensitivities? In addition to numerous sound-producing organs and sound types, several fish taxa have evolved accessory hearing structures which result in a diversity of hearing abilities. However, the functional significance of this diversity remains unclear. Comparative studies revealed that sound characteristics do not always match hearing sensitivities. The conclusion is therefore that the selective pressures involved in the evolution of this diversity were other than those serving to optimize acoustic communication.

Key words: Sound-generating mechanisms, Vocalizations, Sound propagation, Acoustic behavior, Communication

INTRODUCTION

Currently, approximately 25,000 living fish species are known, and several thousand of these are assumed to be vocal. Although fishes most likely represent the largest group of sound-producing vertebrates, our knowledge and level of analysis is incomparably weaker in this group than in

amphibians, birds and mammals. We do not know how many of the 482 families described (Nelson 1994) are vocal or even know how many sound-generating mechanisms exist.

This chapter will begin with a short description of the diversity of sound-producing mechanisms and vocalizations and discuss to what degree acoustic signals are adapted for sound transmission over longer distances. Fishes vocalize in numerous behavioral contexts already known from higher vertebrates such as aggression and courtship; nonetheless, several differences are apparent. Advertisement calls (songs) - acoustic signals used for long distance communication - are widespread in amphibians, birds and insects but have only occasionally been described in fishes. This limits the use of playback techniques for studying the function of vocalizations and is a major reason why our current knowledge of the communicatory significance of fish sounds is very limited.

Is sound production optimized for acoustic communication? The diversity of sonic organs and vocalizations is paralleled by a diversity in the auditory periphery, inner ear structures and hearing abilities in fishes (see other chapters of this volume). It is unclear, however, if these two processes evolved in correlation to each other. Several data contradict the intriguing notion of a correlation. Therefore, the remainder of this chapter will attempt to shed new light on the evolution of hearing sensitivities by analyzing their correlation to sound characteristics.

SOUND-GENERATING MECHANISMS

Teleost fishes have evolved a diversity of sound-generating organs. Interestingly, none is based on vibration of membranes in an airflow, the main principle of vocalization in frogs, birds and mammals including whales (larynx, syrinx). No generally accepted classification of sound-producing mechanisms is available for fishes (Schneider 1961, 1967; Tavolga 1964, 1971; Zelick et al. 1999; Fine & Ladich 2003). Well known sonic mechanisms are swim bladder vibrating and stridulatory mechanisms, several other mechanisms exist although, including pectoral fin plucking or pectoral girdle vibrations. It should also be noted that the sonic mechanisms in several well-known sound producers such as gobies, pomacentrids and loaches remain unknown (Valinsky & Rigley 1981).

Swim bladders are typically put into vibration by rapid contractions of sonic or drumming muscles. These muscles have fibers that either attach at both ends of the swim bladder such as in toadfishes, triglids or certain cods (intrinsic type; Hawkins & Myrberg 1983; Hawkins 1993; Bass & Baker 1991) or fibers that originate on another structure such as the skull or vertebral processes and insert on the swim bladder (extrinsic type) (Fig. 1). In pimelodine catfishes, the sonic muscles originate at the transverse process of the fourth vertebra and insert on the rostral and ventral surface of the swim bladder, thus covering it entirely (Ladich & Bass 1998; Ladich 2001). In the tigerperches (family Teraponidae), a pair of short muscles originate on the occipital region of the skull and insert on the anterior-dorsal surface of the swimbladder (Schneider 1964a). Sonic muscles have a more indirect attachment to the swim bladder in a number of distantly related teleost groups. For example, ariid, doradid and mochokid catfishes have drumming muscles that first insert on a thin bony plate (elastic spring),which is then attached to the swim bladder (Tavolga 1962; Abu-Gideiri & Nasr 1973; Kastberger 1977; Ladich & Bass 1998;
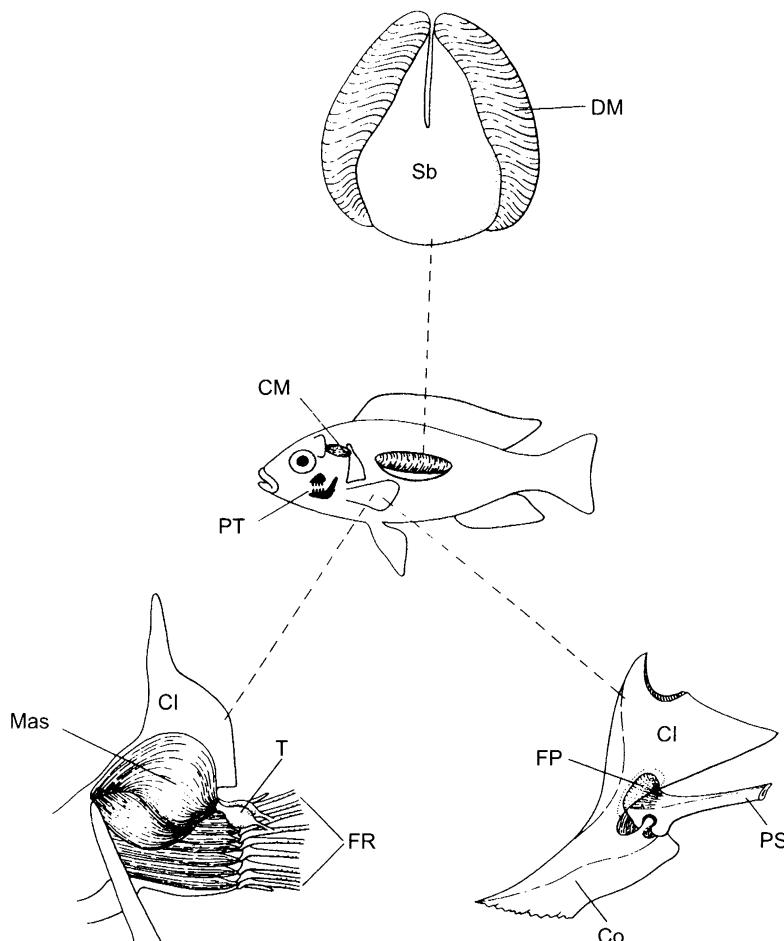
Fig. 1 Sound-generating mechanisms in fishes. Upper: Dorsal view of the intrinsic drumming muscles (DM) embedded in the wall of the swim bladder (Sb) in the toadfish *Porichthys notatus*. Center: Pharyngeal teeth (PT) are the most likely sound source in haemulids, cichlids and centrarchids. Sculpins vibrate their pectoral girdles via the cephaloclavicular muscle (CM). Lower left: Tendon plucking mechanisms of the croaking gourami *Trichopsis vittata*. Lower right: Pectoral stridulatory mechanism in the pimelodid catfish *Rhamdia sebae*. Abbreviations: Cl - cleithrum, Co - coracoid, FP – friction process, FR – fin rays, Mas - superficial adductor muscle, PS – pectoral spine, T – enhanced fin tendons (drawings by H.C. Grillitsch; after Ladich 1991).

Fine & Ladich 2003). This elastic spring mechanism varies in the origin of the sonic muscles as well as in the shape of the elastic spring. Rapid contractions of the sonic muscles set the elastic spring and swim bladder wall into vibration. In squirrelfish (family Holocentridae), a bilateral pair of extrinsic muscles attach to the skull near the auditory bulla and then extend across the upper flattened part of the first two ventral ribs; these are firmly attached to the swim bladder and end in ribbon-like tendons just in front of the third rib (Winn & Marshall 1963). In yet other taxa, swim bladders are vibrated by flat tendons surrounding them either dorsally or

ventrally. For example, among drumfish (family Sciaenidae), the sonic muscle fibers originate from the left and right side of the abdominal musculature and are attached to a broad central tendon that extends between the muscles and dorsally crosses the swim bladder (Schneider & Hasler 1960; Ono & Poss 1982). In piranhas (family Characidae), on the other hand, sonic muscles originate on vertebral processes and insert in a tendon which surrounds the bladder ventrally (Markl 1971).

Stridulatory mechanisms involve the rubbing of bony elements such as teeth or fin rays against each other; this sonic mechanism is similar to that found in most crustaceans and insects (Meyer-Rochow and Penrose 1976, Aiken 1985). Numerous catfish families possess enhanced pectoral fins rays (spines) with a series of ridges on a dorsal process of their proximal end (Fig. 1) (Fine et al 1999; Fine & Ladich 2003). Rubbing these ridges against a groove of the pectoral girdle yields series of short pulses (Pfeiffer & Eisenberg 1965; Ladich 1997; Heyd & Pfeiffer 2000). A stridulatory apparatus of the dorsal fin has been described in the triggerfish, where the first three fin rays are pressed against their base and moved during sound production (Schneider 1961). Less specialized stridulatory mechanisms include rubbing of pharyngeal and other teeth in connection with non-feeding activities such as agonistic behavior. The best known sound producers of this group are grunts (family Haemulidae) (Tavolga 1971). Pharyngeal teeth stridulation is attributed to several additional fish families that produce burst-like sounds such as carangids, centrarchids or cichlids, although the supporting evidence remains sparse (Moulton 1958; Lanzing 1974; Ballantyne & Colgan 1978).

Another unique sonic mechanism is found in croaking gouramis (family Belontiidae), which generate pulsed sounds when snapping two stretched, enhanced pectoral tendons over bony elevations of the fin rays (Fig. 1) (Kratochvil 1978). This mechanism is similar to the plucking of guitar strings and is activated during rapid pectoral fin beating. Sculpins (family Cottidae) lack swim bladders but produce series of sounds or growls by vibrating the entire pectoral girdle. This is mediated via sonic muscles which originate on the skull, insert on the dorsal element of the pectoral girdle, and rapidly pull the girdle towards the skull (Fig. 1) (Barber & Mowbray 1956; Ladich 1989; Bass & Baker 1991).

SOUND CHARACTERISTICS

In contrast to other vertebrate classes, fishes produce short, mostly pulsed sounds with a large variation in spectral and temporal content.

Drumming sounds generated by swim bladder-vibrating mechanisms are tonal and characterized by their low frequencies and harmonic content. The fundamental frequency of drumming sounds reflects the muscle contraction rate, which varies from 80 Hz to over 200 Hz. The dominant frequency of sounds usually corresponds to the fundamental frequency or lies within the second or third harmonic or even switches between harmonics (Bass et al. 1999; Ladich 1997, 1999) (Fig. 2A). These low frequencies contrast with other fast-contracting sonic muscles such as the tymbal muscle in cicadas, where contraction rates of 120 Hz result in peak frequencies of 4.3 kHz due to a frequency multiplier system (Bennet-Clark 2001). Most likely

due to a lack of appropriate vibrating membranes or resonating structures, fishes are unable to produce high frequency (> 1 kHz) tonal sounds.

The duration of drumming sounds and their pulse repetition rate can differ in closely related families or species or even depend on the behavioral context. The fundamental frequency in pimelodid catfish is higher than in doradids (165 - 177 Hz vs. 96 - 114 Hz). The duration of swimbladder sounds recorded in distress situations varied from a few milliseconds to 1.5 seconds. The mean duration was more than ten times longer in *Pimelodus pictus* than in *P. blochii* (445 ms vs. 40 ms) (Ladich 1997). The calls of gadoid species could be distinguished by differences in temporal structures. Knocks and grunts uttered by the haddock *Melanogrammus aeglefinus* can be separated from the cod *Gadus morhua* by their shorter duration and fewer pulses. Both have much slower pulse repetition rates than the lythe *Pollachius pollachius* (Hawkins & Rasmussen 1978). Among mormyrids, weakly electric fishes from Africa, *Pollimyrus adspersus* and *P. isidori* differ distinctly in their pulse repetition rate (56 vs. 44/s). In contrast, complex tone bursts (moans) differed substantially in their peak frequencies (249 Hz vs. 332 Hz) (Crawford et al. 1997).

Numerous species are able to modify their drumming sounds. One of the largest varieties is found in the mormyrid *P. adspersus*, which emits five different types of sounds (moans, hums, growls, hoots and pops) that are used in different contexts (Crawford et al. 1986). The midshipman *Porichthys notatus* produces long-duration hums (>1 min), short duration grunts (ms) and growls (ms to min) which differ in their fundamental frequency (Bass et al. 1999). In the piranha *Serrasalmus nattereri* two types of drumming sounds can be distinguished when the fish are held by hand, namely barks with pulse rates of 80 - 150 Hz and honks with much lower pulse rates (5 - 20 Hz) (Kastberger 1981).

The drumming of some families such as pimelodid and doradid catfishes are frequency modulated (Ladich 1997). Although common in birds and many mammals, frequency modulation is relatively unusual in fishes (Fine et al. 1977). Long spawning calls of the male haddock *Melanogrammus aeglifinus* exhibit distinct frequency modulation (Hawkins & Rasmussen 1978); the mormyrid *P. adspersus* modulates the frequency of moans in either an upward or downward direction (Crawford et al. 1986).

While the muscle contraction rate determines the fundamental frequency and harmonic content of sound in catfishes, characids, toadfishes and cods, this is obviously not the case in species possessing other swim bladder vibrating mechanisms or when short pulses were generated. In two catfish species, *Platydoras costatus* and *Pimelodus blochii*, series of short drumming sounds (6 - 8 ms) which lack any harmonic content were emitted (Ladich 1997). Similarly, sounds of sciaenids are short and show a broad spectrum with frequencies between 150 - 1000 Hz; largest relative amplitudes are in the range of 250 - 600 Hz (Schneider & Hasler 1960; Connaughton et al. 2000). Thus, the predominant frequencies do not reflect the muscle contraction rate, which is approximately 25 - 30 Hz. Juvenile tigerperches (*Terapon jarbua*) emit two types of sounds – drumming sounds and threatening sounds of higher intensity which do not show harmonics (Schneider 1964a).
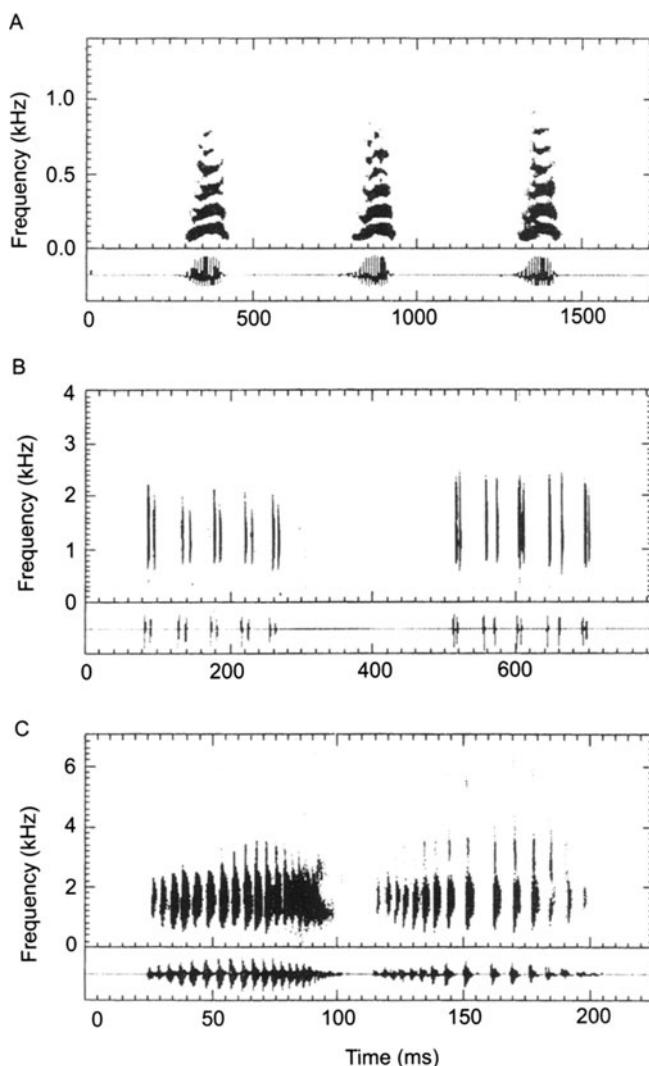
Fig. 2 Sonograms and oscillograms of fish sounds. (A) Three drumming sounds of the piranha *Serrasalmus natereri* uttered when caught (filter bandwidth: 30 Hz). (B) Croaking sounds emitted by two fighting males of *T. vittata*. Sounds of males clearly differ in the pulse period within double pulses (filter bandwidth: 200 Hz). (C) Stridulatory sounds produced by the doradid catfish *Platydoras costatus* in distress situations. Sounds are generated during abduction and adduction of pectoral spines (filter bandwidth: 500 Hz). Note the different X- and Y-axis ranges in A, B and C.

Stridulatory sounds are essentially series of short wide-band pulses or bursts with no harmonic structure. Sounds produced by grinding of pharyngeal teeth are burst-like and have energies mainly concentrated at a few hundred Hz, with some components extending continuously to several kHz. The stridulatory sounds of haemulids (grunts) show predominant frequencies between 100 and 500 Hz (Fish & Mowbray 1970). In cichlids, the main energies of

stridulatory sounds are below 500 Hz (Myrberg et al. 1965; Rowland 1978), although some sound energies extend up to 10 kHz (Nelissen 1978). Similarly, pharyngeal sound energies of centrarchids range up to 12 kHz (Ballantyne & Colgan 1978). Stridulatory sounds produced by rubbing pectoral spines in sockets of the shoulder girdle consist of series of separate pulses with higher dominant frequencies than found in pharyngeal sounds (Fig. 2C). Signals emitted during abduction and adduction of pectoral spines in catfishes have peak frequencies concentrated at or above 1 kHz, and durations varied from 30 - 100 ms (Pfeiffer & Eisenberg 1965; Ladich 1997; Pruzsinszky & Ladich 1998; Kaatz 1999; Ladich 1999; Fine et al. 1999; Heyd & Pfeiffer 2000; Fine & Ladich in press). Duration and intensity apparently differ between families. Among tropical catfish families, adduction sounds were similar to abduction signals in doradids, shorter and of lower sound pressure in mochokids, and totally lacking in pimelodids (Ladich 1997) (Fig. 2B).

The sound characteristics of fish that possess yet other sonic mechanisms reveal further diversity of vocalizations. The sounds of croaking gouramis (genus *Trichopsis*) are built up of regular series of double pulses, each pair produced by one pectoral fin (Kratochvil 1978). Differences exist between the three species of *Trichopsis* in the number of double pulses, pulse periods and dominant frequencies (Ladich et al. 1992a) (Fig. 2B). Damselfish (family Pomacentridae) produce species-specific pulsed sound by an unknown mechanism with main energies between 300 and 700 Hz (Spanier 1979; Lobel & Mann 1995).

The knocking sound of loaches (family Cobitidae) are broad-band, with main energies concentrated at the lower end of the spectrum (< 500 Hz) in the orange-finned loach *Botia modesta* (Ladich 1999). Sculpins (family Cottidae) emit single knocks or series known as growls, whereas polypterids produce thumps and moans of longer duration, all at low frequencies (Ladich & Tadler 1988; Ladich 1989). Similarly, gobies generate pulsed or harmonic signals sometimes below 100 Hz (Ladich & Kratochvil 1989; Lugli et al. 1995).

The dependency of sound characteristics on body size varies across sound-generating mechanisms (Fine & Ladich 2003). In general, dominant frequencies are not correlated to body size when they clearly reflect muscle contraction rates such as in swim bladder vibrating sounds of doradid and pimelodid catfishes (Ladich 1997). In contrast, a clear relationship between dominant sound frequencies and body size has been found in species that produce pulsed sounds. Thus, in all three species of croaking gouramis, the dominant frequencies of signals emitted by the pectoral fin tendons are inversely correlated to body mass (Ladich et al 1992a). Furthermore, Henglmüller & Ladich (1999) and Wysocki & Ladich (2001) show that the dominant frequencies of croaks decreased during ontogenetic development in *Trichopsis vittata* from about 3 kHz to 1.5 kHz. In damselfish, main energies of chirping sounds reflect a clear inverse relationship to body length (Myrberg et al. 1993; Lobel & Mann 1995). In the latter cases it is assumed that the relationships are mediated by the resonance frequency of air-filled cavities such as swim bladders or air-breathing chambers in anabantoids. This phenomenon might also explain the decrease of frequencies with increasing size in species that produce short pulses using swim bladder muscles such as in drums, tigerperches and mormyrids (Schneider 1964a; Crawford et al. 1997; Connaughton et al. 2000).

ECOLOGY OF SOUND PRODUCTION

Did the diversity of sound-generating mechanisms and sound types evolve as an adaptation to environmental constraints, to distances over which acoustic information has to be transmitted, to existing hearing sensitivities (sensory exploitation hypothesis - Ryan & Keddy-Hector 1992), or are they the result of existing morphological preadaptations for sound production? The limitations of sound frequencies to lower ranges in numerous species might be seen as an adaptation to restrictions in auditory sensitivities, especially in species possessing no morphological specializations for hearing enhancement such as toadfishes, gobies, cichlids, sciaenids or polypterids; they detect only the kinetic component of low frequency sounds in the vicinity of a sound source. Interestingly, in very shallow waters such as tidal pools, lakes and shallow streams, where most of the sonic fish species breed and defend territories (Ladich 1997), the propagation of low-frequency sounds is heavily restricted. The frequency below which sound transmission is negligible is referred to as the cut-off frequency. Depending on the composition of the bottom substrate, the cut-off frequency at 1 m depth is 300-1000 Hz (Rogers & Cox 1988; Schellart & Popper 1992; Boatright-Horowitz et al. 1999). Toadfish commonly call in 1 m deep water and the fundamental frequency of the boatwhistle call is 200 Hz. Fine & Lenhardt (1978) broadcasted low-frequency acoustic signals (tones, noise, courtship calls) in 1 m deep water over medium to fine sandy bottoms and observed that, for the pure tones, transmission loss was greatest within the first 3 m from the transducer. In boatwhistles, the fundamental frequency was absorbed more quickly than its second harmonic, which is unlikely to be heard in this species. At a distance of 5 m, the signals were no longer above the background noise levels. Crawford et al. (1997) found that the fundamental frequency of the sound of the weakly electric fish *P. isidori* is not likely to propagate well in 2 m deep freshwater flood plains in West Africa. Propagation tests of pulsed damselfish sounds performed in 7 m deep water revealed that it is unlikely that fish can detect sounds over more than 11 m away from the speakers (Mann & Lobel 1997). All these data indicate that fish sounds function over a short distance and that they attenuate and degrade rapidly in shallow waters. It is unlikely that fishes can communicate acoustically over longer distances; so far, no field experiments are available which show that communication can take place beyond 10 m. Numerous behavioral studies clearly demonstrate that fish communicate acoustically at much shorter distances, mostly after a conspecific has been detected visually (Myrberg 1981; Ladich 1997). Fishes such as croaking gouramis, sculpins, gobies and catfishes vocalize during agonistic encounters and courtship when they are 1 - 5 cm from the opponent or mate (Ladich 1989; Ladich & Kratochvil 1989; Ladich et al. 1992a, b; Pruzsinszky & Ladich 1998).

Several groups such as pomacentrids, gouramis and catfishes produce pulsed sounds with main energies above 500 Hz or even 1000 Hz (Myrberg & Spires 1980; Ladich 1997; Ladich & Yan 1998) and are also able to detect higher frequency sounds. These groups should be able to communicate over longer distances because those sound frequencies are most likely above the cut-off frequency in shallow water. However, it is unknown whether such differences in the communication distance exist between groups using different frequency ranges. Furthermore, it remains unknown whether the diversity in sound types evolved as an adaptation to different

sound propagation characteristics of various habitats. This has been observed in other verte-brates such as birds and primates, which utilize different sound channels and signal types in order to optimize transfer of information in differently structured habitats (Brown & Waser 1988; Krebs & Davies 1993). Great tits in dense forests sing songs with a narrower range of frequencies, lower maximum frequency and fewer notes than the songs of open country birds (Hunter & Krebs 1978). No such an adaptation has yet been demonstrated in fish.

Therefore, the variety of song types and frequency ranges cannot be explained based on different demands on communication distance or habitat acoustics. Limitations of hearing or-gans and/or sound-generating mechanisms might be better candidates to explain low frequency acoustic signaling in fishes. As has been pointed out earlier, sound-producing organs in fishes are mainly based on rapid muscular vibrations of swim bladders (Bass & Clark 2002) and pecto-ral girdles or on rubbing, plucking or friction processes resulting in broad-band pulsed sounds including low frequency components in all cases (Fish & Mowbray 1970; Ladich 1997). In no case are fishes able to generate high frequency tonal sounds such as dolphins or whales, although this might be advantageous regarding the habitats occupied.

VOCALIZING BEHAVIOR

Fishes produce sounds in a variety of behavioral contexts, especially during agonistic interac-tions, courtship and spawning. A great part of our knowledge derives from vocalization in dis-tress situations, i.e. when they are disturbed, caught or hand held or in some way manipulated (Fish et al. 1952; Fish & Mowbray 1970; Fine et al. 1977; Myrberg 1981). Incidental sounds have been described during rapid air release from the swimbladder, during motion of fish through the water (hydrodynamic sounds) or during feeding (Dijkgraaf 1941, Tavolga 1971). Because it remains questionable whether the latter have any functional or social significance, they will not be dealt with further here.

Can emission of sounds always be regarded as part of a communicatory process? Indepen-dent of which definition of communication we apply, the transfer of information from a sender to a receiver has to be demonstrated (Myrberg 1981; Markl 1985). Communication is usually assessed by a change in the behavior of the recipient. Note, however, that the behavioral (or perhaps physiological) significance of only a small fraction of fish sounds is known. Describing sound production in a certain context often implies a functional significance (e.g. threatening sounds) but does not confirm it. In order to separate description from function, the first part of this section is mainly descriptive, whereas the second part discusses the communicatory signifi-cance of sound production.

Aggressive encounters in which sounds are produced usually start after the opponent has been detected visually. In sculpins and gobiids, for instance, visual displays consist of fin spread-ing, erecting of opercular covers or lowering of the branchial membrane and darkening (Ladich 1989; Ladich & Kratochvil 1989). Threatening behavior may result in parallel or antiparallel displaying and circling such as in croaking gouramis (Fig. 3). Sound production accompanying visual displays may cause characteristic body movements, e.g. nodding in the European river bullhead *Cottus gobio* (Ladich 1989) or rapid pectoral fin beating and whole
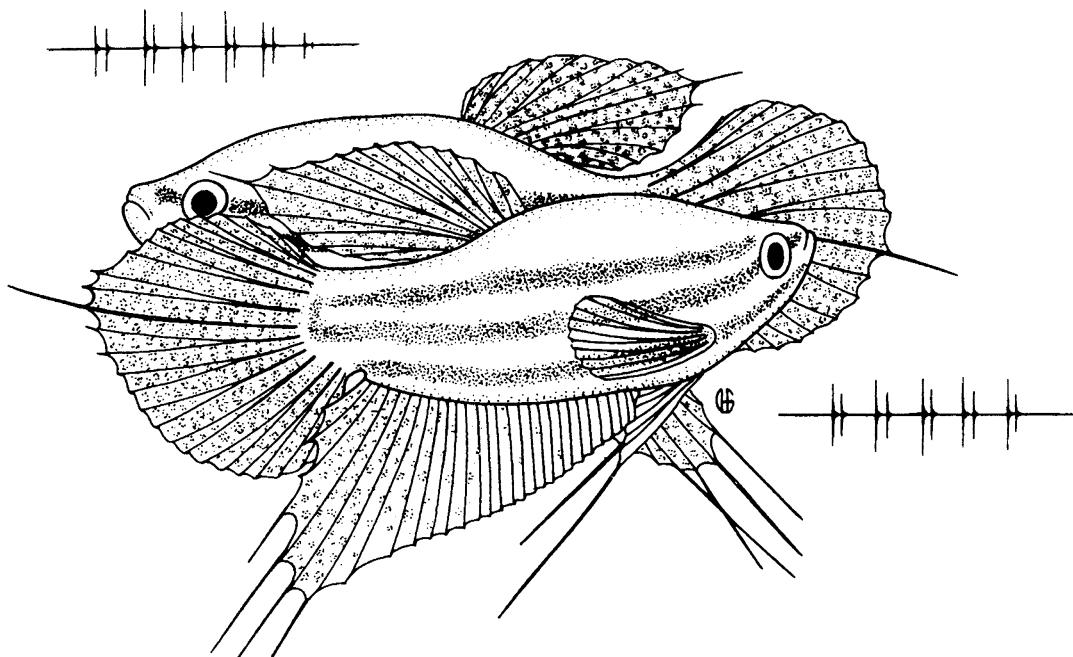
Fig. 3 Fighting displays of two vocalizing croaking gouramis (*T. vittata*). Rivals produce acoustic signals alternately while beating pectoral fins rapidly, spreading unpaired fins and circling in a head-to-tail position. Inserts show oscillograms of croaking sounds emitted by the opponents (drawing by H.C. Grillitsch).

body shaking in *T. vittata* (Ladich et al. 1992a, b). If these displays do not effect the retreat of an opponent, the agonistic encounters may further escalate to more costly behavioral elements such as biting. In a recent survey, Ladich (1997) listed representatives of more than 30 families of fishes in which sound production has been described during agonistic interactions. This listing includes one non-teleost family (Polypteridae) and representatives of several teleost orders: osteoglossiforms, otophysines (characiforms, cypriniforms, siluriforms), gadiforms, batrachoidiforms, beryciformes, scorpaeniformes, numerous families of perciformes and tetraodontiforms. This list will likely become much longer in the future because it is assumed that most of teleost fishes are vocal.

Several species produce one type of sound but others are capable of uttering different types of acoustic signals during agonistic interactions. In *Polypterus* threatening behavior consists of erecting all dorsal fins and emitting a series of thumps. If the smaller individual does not flee, biting results. The fleeing individual usually produces moans, a flight or submissive sound (Ladich & Tadler 1988). Schneider (1964b) described threatening sounds and fighting sounds in the damselfish *Amphiprion* sp. The noise-like threatening sounds are loud and emitted to threaten other fish over longer distances, whereas croak-like fighting sounds are produced near the rival.

During reproductive activities, sounds are emitted in a variety of behavioral contexts whose bondaries are fluent. Males utter advertisement calls without visual contact to females; the obvious function is to attract potential mates and probably to repel rivals. Classic examples are the hums and boatwhistles of the midshipman and oyster toadfish (Winn 1967; Bass et al. 1999). Chirping sounds of the bicolor damselfish *Eupomacentrus partitus* also fall into this category (Myrberg et al. 1986). Contrary to these far-distance signals courtship sounds might be regarded as near-distance signals produced when mates are interacting at close distances prior to spawning. Courtship sounds have been described in numerous families (Myrberg 1981) including cyprinids (*Notropis analostanus* - Stout 1963), mormyrids (*Pollimyrus* spp. - Crawford et al. 1986), gobiids (Lugli et al. 1997), pomacentrids (Kenyon 1994), and callichthyid catfish (Pruzsinszky & Ladich 1998). These sounds are typically emitted by males when females approach nesting sites of males; they are accompanied by a variety of visual displays such as erecting fins, spreading opercula, undulating body movements and leading females to the nest. In the catfish *Corydoras paleatus*, males clasp female barbels with one pectoral spine while stridulating with the other (Pruzsinszky & Ladich 1998). Advertisement and courtship calls stop as soon as females enter nests, after which spawning sound may occur. Males of the goby *Padogobius martensi* produce tonal sounds as long as the female is outside the nest. They switch to drumming sounds after the female enters and spawning begins, ceasing only after the female departs the nest hollow (Lugli et al. 1997). Spawning sounds are not always uttered by males. In the croaking gourami *T. vittata*, females produce low-intensity purring sounds between spawning acts (Marshall 1966; Ladich pers. obs.).

Besides agonistic and reproductive activities, some sounds seem to be produced during schooling, although this needs to be examined more closely. The gudgeon *Gobio gobio*, a small European cyprinid which neither defends territories nor attacks conspecifics within shoals, regularly produces high-pitched creaking sounds when disturbed, touched or hindered by conspecifics. Thus, it is assumed that sounds produced by fishes in aggregations are mainly aggressive and help to maintain individual distances between members of a shoal (Ladich 1988).

FUNCTIONAL SIGNIFICANCE OF SOUNDS

The functional significance of most fish sounds has not been determined. This is partly due to the large effort that working underwater in the field entails and due to methodological difficulties in separating the effect of acoustic signals from signals of other modalities. Three different approaches have been used to deal with the latter problem: playback of sounds, muting experiments and correlative investigations.

Sound playbacks have been successfully applied when vocalizations serve as long-distance signals without the involvement of other signal modalities. Exemplary cases include analyzing the function of advertisement calls in frogs and insects and of the song in birds (McGregor 1992). In fishes, where near-distance signals dominate, only a few playback studies were performed successfully without additional stimuli. Myrberg and colleagues showed in a series of playback studies in the field that the chirping sound of the coral reef fish *P. partitus* fulfills

several functions. Myrberg et al. (1986) demonstrated that females orient towards sound sources (loud speakers) and choose males based on the dominant frequencies of sounds. Spanier (1979) showed that sound of four damselfish species differs slightly in the pulse number and period and that these differences function in species recognition. Furthermore, Myrberg & Riggio (1985) demonstrated that all males within a damselfish colony recognize neighbors individually solely by acoustical means. They respond more aggressively to sound playbacks of unknown intruders as compared to known neighbors. Similarly, toadfishes (family Batrachoididae) emit advertisement calls and are suitable for playback studies. Males of the oyster toadfish *O. tau* increased their calling rate when boatwhistles were played back at a rate of 18 to 30 per minute (Winn 1967). Males seem to advertise their resource-holding potential against other males; this might play an important role in the establishment and maintenance of territories. In a recent series of experiments McKibben & Bass (1998) showed that positive phonotaxis could be elicited in female midshipman *P. notatus*: the females preferred the more intense of two signals and the frequency preference was temperature dependent. This is in contrast to Myrberg et al.'s (1986) findings showing that female damselfish prefer the lower frequency sound, which indicated larger males.

Sounds of males may influence gonadal activity in females similarly to the song of numerous birds. To date our knowledge is limited to one scientific abstract dealing with fishes. Playbacks of sounds of male mouthbrooding cichlids *Oreochromis (= Tilapia) mossambicus* apparently stimulate gonadal development of conspecific females because these spawned earlier than control groups (Marshall 1972).

Agonistic sounds usually need signals of other modalities to elicit appropriate responses (Ladich 1997). Schwarz (1974) played back low growling sounds to pairs of *Cichlasoma centrarchus* that were acoustically but not visually isolated from each other. Playbacks markedly lowered the number of highly aggressive encounters males directed at either their male or female partners and therefore this sound functions to inhibit aggressive behavior in the recipient. Similar observations were described by Rigley & Muir (1979) in the brown bullhead *Ictalurus nebulosus* when a second fish was introduced to an individual holding a territory. Playback of rachet sounds decreased the number of attacks by the resident. Therefore, rachet sounds of the intruder fish may be an 'appeasement' display. On the other hand, Stout (1963) demonstrated that reactions to sounds depend on rank within a dominance hierarchy. In these tests with isolated male satinfin shiners *Notropis (= Cyprinella) analostanus* given mirrors to provide visual stimuli, playback of a rapid series of knocks stimulated aggressive behavior by the dominant male but inhibited aggressive displays in a submissive male. Visual and acoustical signals do not have to be presented simultaneously in order to obtain results. Aggressive sounds are capable of eliciting approach responses toward the loudspeaker area in the male goby *Padogobius martensi* even when the mirror was removed 10 to 15 minutes earlier (Lugli 1997).

Muting experiments circumvent the artificial presentation of acoustical signals and compare differences between muted fishes and unaltered controls. Muting studies were seldom performed because they require detailed knowledge of the sound-producing mechanism as well as a procedure that does not impair other behaviors. Valinsky & Rigley (1981) muted juvenile

skunk loaches *Botia horae* by blocking opercular movements with a steel wire, which obviously inhibited the production of click sounds. Muted fish performed lateral displays at higher rates but attacked at much lower frequencies than controls and were unable to chase intruders from their shelters. However, it is unclear to what degree the hindering of opercular movements influenced the results. The croaking gourami *T. vittata* produces high amplitude sound using enhanced pectoral fin tendons (two out of eleven are enhanced), which can be cut without inhibiting the calling movement (= rapid pectoral fin beating) (Ladich et al. 1992b). Muted and unaltered, previously isolated males were paired in two different asymmetry groups and the outcome of dyadic encounters was analyzed. In pairings in which the size difference was pronounced, larger fish won more contests than smaller ones, regardless of vocalizing ability. When size asymmetry was small, however, intact males won more contests than muted fish. These results indicate that size and most likely territoriality are the main factor to win contests and that acoustical signals gain importance when asymmetries in the previous factors are small.

Correlative studies attempt to analyze the significance of sound without manipulating the fish. The outcome of reproductive behavior can be correlated with number of sound or with certain sound characteristics that better explain the results than do other displays or morphological parameters. Schuster (1986) observed that the defense of territories in the dwarf gourami *Colisa lalia* was more effective when attacks were accompanied by sounds than without. In 31% of the sound attacks, intruders fled quickly over longer distances; the value was only 5% in no-sound attacks. Quantity and quality of sound serve as predictors of fighting ability in several fish species. In the European river bullhead *Cottus gobio*, calling frequency is highly correlated with body length, success in agonistic encounters, and territory dimensions (Ladich 1990). Correlations revealed that fighting success depended more on vocalization than on body size. In a subsequent study, Ladich (1998) showed that in *T. vittata* the relative fighting ability was assessed by acoustic signals and that sound characteristics influenced winning. Besides body weight, sound pressure level and dominant frequency were predictors of the outcome of pair-wise contests, while traits not correlated to size such as number and duration of displays did not influence winning. In accordance with the main predictions of the assessment models, the contest duration (costs) increased with the decrease in asymmetry in body length as well as sound pressure level. These results suggest that *T. vittata* and fishes in general settle conflicts without damaging combat by assessing asymmetries in different components of their resource holding potential such as body weight and length, which may reliably be signaled by acoustic and visual assessment signals.

Communication is usually regarded as a process of information transfer in which both the sender and the receiver benefit. However, sounds are often perceived by fishes for whom the sounds were apparently not intended. This process, termed interception by Myrberg (1981), can take place intra- as well as interspecifically. Kenyon (1994) observed that males of the bicolor damselfish *E. partitus* regularly moved towards the source of the low-intensity grunts, which are usually emitted by another male prior to spawning with a female. This can clearly be a disadvantage for the sender. Lower sound intensity during mating in *T. vittata* probably reduces interception by conspecific males or even predators. The degree to which calling poses a predation threat for vocalizing fishes has not been examined so far but has been confirmed in other

vertebrates such as frogs (Tuttle & Ryan 1981). Data from several regions indicate that fish producing loud sounds comprise the bulk of the diet of bottlenose dolphins, suggesting that dolphins capture their prey by passive listening (Barros & Myrberg 1987). Luczkovich et al. (2000) found that dolphins whistles lower the loudness of mating choruses in the silver perch *Bairdiella chrysoura* (family Sciaenidae). This might be regarded as a counter strategy against predation threat in vocalizing fish, although it currently remains unknown whether sciaenids are able to detect sounds within the frequency range of dolphin whistles (3-4 kHz).

CORRELATION BETWEEN SOUND PRODUCTION AND DETECTION

The large diversity of sound-generating mechanisms results in the production of many different types of sounds. This raises the question whether the auditory system of fishes is adapted to detect conspecific sounds and is thus optimized for intraspecific acoustic communication. Fishes are generally able to detect the kinetic component of sounds and are therefore limited to sensitivity at low frequencies of a few hundred hertz and to the near field of a sound source (hearing generalists; Popper & Fay 1999). Several taxa are able to exploit the displacements produced by contractions and expansions of a gas bubble (e.g. swim bladder) in a sound pressure field. This considerably improves the auditory sensitivity (lowering of thresholds, extension of hearing range, far field sound detection), especially when morphological structures form a close connection between the pulsating air-filled cavity and the inner ear (hearing specialists) (Fig. 4). In otophysans, Weberian ossicles transmit oscillations of the swim bladder to the inner ear, whereas in mormyrids, anabantoids and other groups air-filled cavities are attached to the inner ear (see other chapters of this volume).
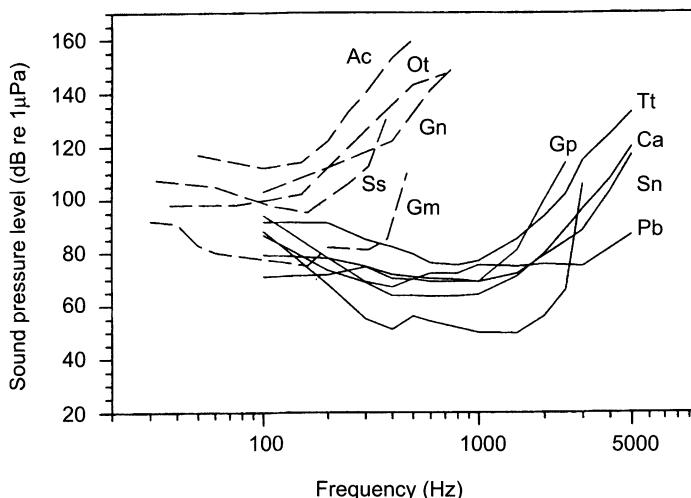
Fig. 4 Auditory thresholds for fishes without known hearing specializations (Ac – *Acerina cernua*, Gm – *Gadus morhua*, Gn – *Gobius niger*, Ot – *Opsanus tau*, Ss – *Salmo salar*) and for hearing specialists (Ca – *Carassius auratus*, Gp – *Gnathonemus petersii*, Mk – *Myripristis kuntee*, Pb – *Pimelodus blochii*, Sn – *Serrasalmus nattereri*, Tt – *Trichogaster trichopterus*). Audiograms after Dijkgraaf (1952), Wolff (1968), Chapman & Hawkins (1973), Hawkins & Johnstone (1978), Coombs & Popper (1979), McCormick & Popper (1984), Fay (1988), Ladich & Yan (1998) and Ladich (1999).

How is this diversity in auditory sensitivities in fishes related to that in sound types? Several studies suggest that sound characteristics such as sound spectra are correlated to the hearing abilities and specializations in fishes. In the damselfish E. *partitus* and the weakly electric fish *Pollimyrus adspersus*, the peak energy of sounds match the audiograms in the region of greatest sensitivity at about 500 to 600 Hz (Myrberg & Spires 1980; Marvit & Crawford 2000). However, these findings cannot be generalized. Low-frequency drumming sounds have been described in numerous hearing specialists (catfishes, characids, mormyrids) and generalists (toadfishes, triglids, drums). Secondly, broadband high-frequency sounds occur mainly in specialists but were also described in non-specialists such as centrarchids (Ballantyne & Colgan 1978). Furthermore, contrary to accessory hearing structures such as Weberian ossicles in otophysines and suprabranchial chambers in anabantoids, sonic organs do not occur in all members of these taxa. Comparing the audiograms of nine representatives of seven otophysine families from four orders revealed major differences in auditory sensitivity, especially at high frequencies (> 1 kHz) where thresholds differed by up to 50 dB (Ladich 1999). These differences showed no apparent correspondence to sound-producing ability because no clear difference between vocal and non-vocal species such as the goldfish were found. The correlation between the spectral content of species-specific sounds and the hearing abilities revealed a clear match in T. *vittata*, a modest match in several catfish species, but also a mismatch in the callichthyid catfish *Corydoras paleatus* (Fig. 5) (Ladich & Yan 1998; Ladich 2000).

This varying degree of correspondence between sound detection and vocalization in two major taxa of specialists together with the lack of a clear difference between vocalizing and non-vocalizing species, indicates that the selective pressure involved in the evolution of diversity of hearing abilities was not to optimize acoustic communication but most likely to detect the entire surrounding auditory scene (Ladich 2000).
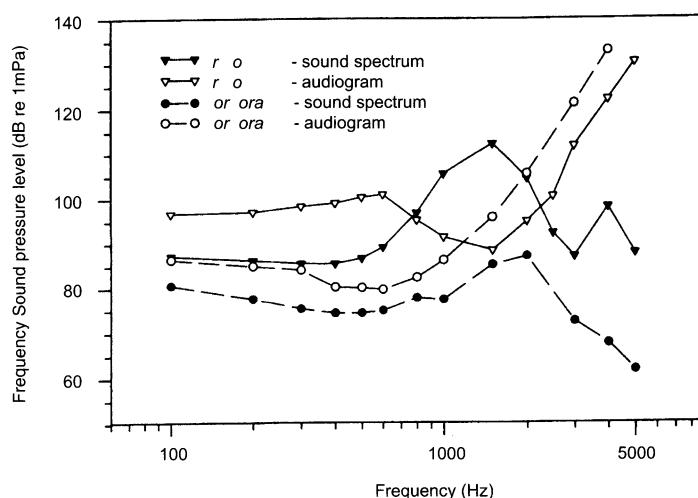
Fig. 5 Audiogram of the croaking gourami T. *vittata* and the callichthyid catfish *Corydoras paleatus* in relation to spectral and intensity characteristics of sounds. Audiograms and sound spectra after Ladich & Yan (1998) and Ladich (1999).

Acknowledgements

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Lateral Line Sensory Ecology

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ABSTRACT

Because all fish experience night, perpetually dark, or turbid waters, there is strong selection for the use of non-visual senses in all fish species. Anatomical diversity hints that the lateral line is one of the most important senses for fishes, and it is put to diverse use. Research on the function of the lateral line has lagged due to poor understanding of hydrodynamics at small scales and lack of this sense in humans, making it difficult to imagine a fish's hydromechanical world. Consequently, much work has focused on the use of an easily generated stimulus, an oscillating sphere. This yields some useful information, but recent work on how potential prey and predator animals move water shows that an oscillating sphere is highly non-biological. Understanding the mechanosensory ecology of an animal requires an understanding of the demands from diverse natural stimuli.

To contribute to an evolutionary future an animal must be good at procuring food, finding mates, and detecting and avoiding or deterring enemies. This puts somewhat contrary demands on the fish in that it must have a sense that can scan as much of its environment as possible for general stimuli, but also have a sense that can investigate details of specific stimuli. The evidence indicates that the lateral line system meets some necessary requirements to be either a scanning or a focused sense, depending on the environmental needs. Superficial neuromasts may be the most sensitive receptors, but they are adversely affected by environmental and self-generated noise. Different types of lateral line canal can filter out different types of hydromechanical noise. At least some fishes can use their lateral line for appropriate positioning relative to conspecifics in a school or prey, or to escape from predators. It is probably used in detecting mates and competitors. How a fish fits its lateral line sense into its ensemble of senses must vary from species to species. Therefore, as studies become more sophisticated, we are likely to discover interesting adaptations and compromises.

Key words: Fish, Fluid mechanics, Lateral line, Mechanosense, Sensory ecology

INTRODUCTION

It is difficult to comprehend the sensory world of animals equipped to be sensitive to signals humans cannot perceive, yet it is essential in order to understand behavior and ecology. For example, understanding bat predator/prey interactions underwent a revolution once the use of ultrasound in echosounding was discovered. Humans have a window into this ability because

we hear echos. The two fish senses most challenging for human understanding are probably the hydromechanical lateral line sense and electrosense. Understanding of electrosense is greater than that of the lateral line in part because of the tools available for measuring electrical fields. Tools for measuring small water currents and our relatively poor understanding of fluid mechanics at small scales has hampered lateral line research. An understanding of the diverse hydrodynamic worlds of fishes leads to an understanding that the lateral line sense is essential to their behavior and ecology.

Perhaps the most useful notion for human understanding of the lateral line sense comes from Dijkgraff's (1963) concept of "distance touch." Humans investigate unseen places by extending their tactile sense with a probe. A rigid probe is more sensitive for this task than a flexible one because the signal is not dampened. For example, anglers trying to discern subtle strikes from snags prefer stiff rods and lines with little stretch. Hairs are inert, flexible, tactile rods whose sensors are in the skin and those modified to be mainly sensory (vibrissae) are stiffer than insulating hairs. The "vibrissae" in distance touch is water, which is more continuous. Its fluidity means it is somewhere between stiff and flexible. For this review, I consider water and a tactile hair to be analogous. Water connects lateral line receptors to other objects. In the case of seals the analogy is very good in that the water extends the range of vibrissae to objects beyond their immediate touch, so they function much like a lateral line system (Dehnhardt et al. 2001).

Factors other than being an alien sense impede a general appreciation of the importance of lateral lines. To the student using fish keys "lateral line" means the trunk canal and this is usually more obvious than head canals or superficial neuromasts. A character of herrings (Clupeidae) used in many keys is the absence of a lateral line. Yet herrings have very elaborate head lateral line canals and the trunk canal, while short, is present and bends ventrally at the anterior part of the trunk. The problem is largely semantic in that receptors outside of canals are generally ignored or given another name by anatomists.

OVERVIEW

Lateral line mediated behaviors are discussed after consideration of the anatomy and physiology, the physics of signals, and functional morphology. The behaviors discussed include swimming and schooling, feeding, predator avoidance, and intraspecies interactions. Most of the behavioral discussion focuses on feeding, where there has been the most work.

For the lateral line, we should not presume that all fishes have the same abilities. Some lateral line systems or parts may be specialized to detect subtle motion, while others are specialized for localization or even "imaging" of the hydrodynamic field, an ability that would allow discrimination of prey types, conspecifics, etc. The best approach is as a naturalist questioning the nature and meaning of diversity. I hope this chapter is a start.

HYDROMECHANICAL SIGNALS

The language for hydromechanical signals is borrowed from other senses, a consequence of not being part of the human sensory repertoire. Signals for the lateral line are typically characterized

by their acoustic spectrum, a concept derived from hearing. This is incomplete because it does not include the fluid's dynamic spatial distribution. The signal received for a prey's feeding or ventilation current varies considerably with the relative position of predator and prey and affects the response distance of the predator (Janssen 1997). More complex are the "footprints" described for swimming by fishes (Müller et al. 1997), copepods, and other zooplankters (Yen and Strickler 1996, Doall et al 1998). The footprints left in water disperse faster than those left in mud, about 30 s for goldfish (*Carassius auratus*, Cyprinidae, Hanke et al. 2000).

Signals generated by animals are used for feeding and/or respiration and locomotion. These flows flush water away from the animal because nutrients and/or oxygen have been extracted or wastes have been added. In locomotion Newtonian mechanics require thrust, i.e. motion of water in the direction opposite the motion of the animal.

Hydromechanical signals to be detected include those from prey, predators, conspecifics and other organisms, obstacles, and stream flows. We generally expect that neither predator nor prey wants to reveal itself and may make efforts to make its signal either less detectable or its source less able to be located (misdirected).

In most lateral line studies an artificial stimulus is used; usually the stimulus is a sphere attached to a vibrator by a rod. This is a simple assembly with equations approximately describing the water flow around the sphere (Fig 1, Kalmijn 1988). However, this stimulus has three problems:

(1) Water *flow around a sine wave oscillating sphere produces acoustic streaming.* Equations predict radial symmetry in oscillating water flow around the axis of oscillation and also reflective symmetry in a plane orthogonal to the axis centered at the sphere's center (Fig. 1, Kalmijn 1988). The equations also predict temporal symmetry in that a particle is expected to oscillate around a point with no net transport (AC flow). However, oscillating spheres and cylinders act as pumps via "acoustic streaming" (DC flow) so there is net transport (Andrade 1931; Andres and Ingard 1953 a, b).

(2) *Mass and spring.* Any rod holding a sphere will have some elasticity so the sphere and rod comprise a mass and spring with its own characteristic frequency. If motion is along the axis of the rod, the usual case, a well-centered sphere's motion will have little deviation from the axis. However, motion orthogonal to the rod can be complex as the driver and resonator components move in and out of phase. Particle streak photography of such a system shows particle movement is erratic.

(3) *Biological signals.* Most potential lateral line stimuli come from the sender moving water past its body to remove wastes or collect nutrients and/or oxygen, or to generate thrust. There would be no reflective symmetry due to the unidirectional (but usually pulsed) flow and because the water flow streamlines at a nozzle taking in water are spheroidal while the outflow streamlines are more conical. Because most animals have dorsal and ventral sides and appendages on one or the other side accelerate the water, there will be bilateral rather than radial symmetry with different flows at the dorsal versus ventral sides (Fig. 1). There is great interspecies diversity in the feeding currents generated by

calanoid copepods, the best studied animals, and each individual can produce a wide variety of hydromechanical signals (Yen and Strickler 1996) none of which resembles an oscillating sphere.

At least one animal, the tube-dwelling decapod (*Callianassa*) apparently does not produce a pulsed flow (Stamhuis et al. 1998). This minimizes the energy expended in pumping, but it may also make the current less apparent to predators.

Another class of signals is generated by a transiting organism. There are two distinctly different signals depending on whether the animal is coasting or actively swimming. For a fusiform coasting animal there is positive pressure at the head (assuming it swims headfirst), negative pressure due to the Bernoulli effect where the body is widest/deepest, and positive pressure at the tail. This is approximately what Dubois et al. (1974) found for bluefish (*Pomatomus saltatrix*). If the animal's size and/or speed are great enough then there will be vortices shed, either in a rhythm (i.e. von Karmen eddies) or somewhat disorganized (turbulence). In this case the wake has pressure variation (vibration). The less streamlined the animal is, the less the pressure distribution is as predicted and the more vibration in the wake. Hence a transiting sphere has much more drag than a fusiform body and generates much more signal than a streamlined animal.

If the animal is swimming then it must generate thrust, meaning the pressure behind the animal is greater than the pressure in front of it (otherwise the animal will not move or move backwards). Even in the simplest case of slow movement (no flow separation) there is a great
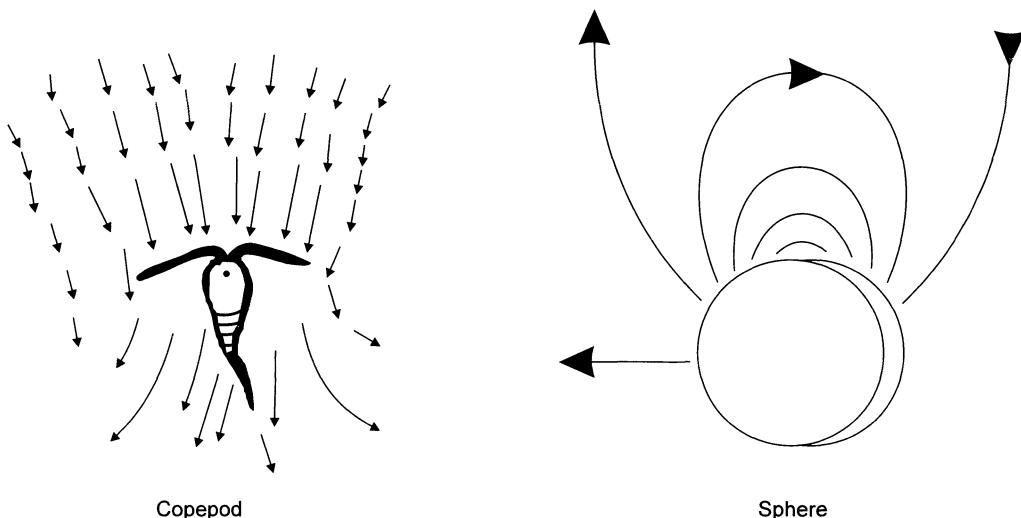
Copepod   Sphere

Fig. 1 Water flows (arrows) past a stationary calanoid copepod and an oscillating sphere. The copepod (*Centropages hamatus*) was tethered and flows measured by tracking particles (redrafted from Hwang and Strickler 2001). For the sphere, flows are from calculations (redrafted from Kalmijn (1988)). The flow around the sphere reverses when the sphere changes direction and flows are radially symmetrical around the axis of motion.

difference in the flow past a towed sphere versus a self-propelled one (Fig. 2, Jiang et al. 2002). The flow field can be very complex and even species specific. The pressure distribution and flows for a fish swimming via pectoral fin flapping (labriform swimming) or dorsal and anal fin flapping (balistiform swimming) will be different than for a fish using caudal propulsion. In Antarctic krill (*Euphausia superba*) metachronal beating of pleopods creates a propulsion jet with pressure pulses at the appendage beat frequency and one or two harmonics (Wiese and Ebina 1995). For calanoid copepods generating a feeding current there is a negative pressure at the head that draws water to its feeding appendages; the water exits in approximately the opposite direction. There are species-specific flows among the calanoid copepods so it is possible that some predators can distinguish between species. *Daphnia* also generates a feeding current, but the strongest signal may be due to the hop and sink swimming generated by the swimming antennae (Kirk 1985).
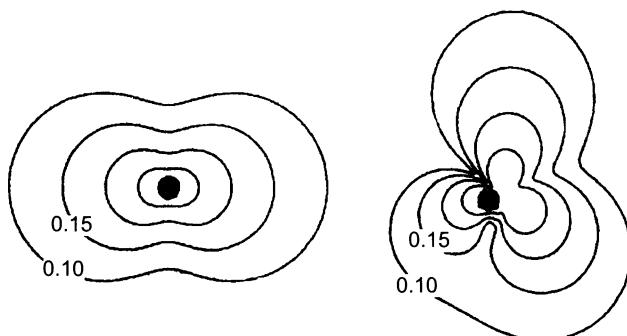
Fig. 2 Water speeds around a towed (left) versus a self-propelled (right) sphere, calculated as a fraction of sphere speed. The lines indicate isometric speeds and numbers are relative speed. Reynolds numbers are small so there is no flow separation. Redrafted from Jiang et al. (2002).

LATERAL LINE ANATOMY

The components of lateral line anatomy considered here include the sensory organ (neuromast) and skin structures that modify the signal it receives. Further anatomical description and neurophysiology are covered by Mogdans et al. (next chapter). The neuromast consists of rather conventional hair cells in which the stereocilia and kinocilia are imbedded in a gelatinous cupula (Fig. 3). The hair cells in a neuromast are aligned along the same axis in one of two orientations so that for any water flow over the neuromast, about half of the hair cells are hyperpolarized and half are depolarized. The number of hair cells increases as the fish grows in mottled sculpin (*Cottus bairdi*, Cottidae, Janssen et al. 1987) and the number of superficial neuromasts can increase with growth. Neuromasts are either on the surface of the skin (superficial neuromasts) or in canals (Fig. 3) but all begin their ontogeny as superficial neuromasts.

The diversity of superficial neuromast and lateral line canal distributions is reviewed in Coombs et al. (1988); some examples are given in Figs. 4 and 5. A typical distribution of canals
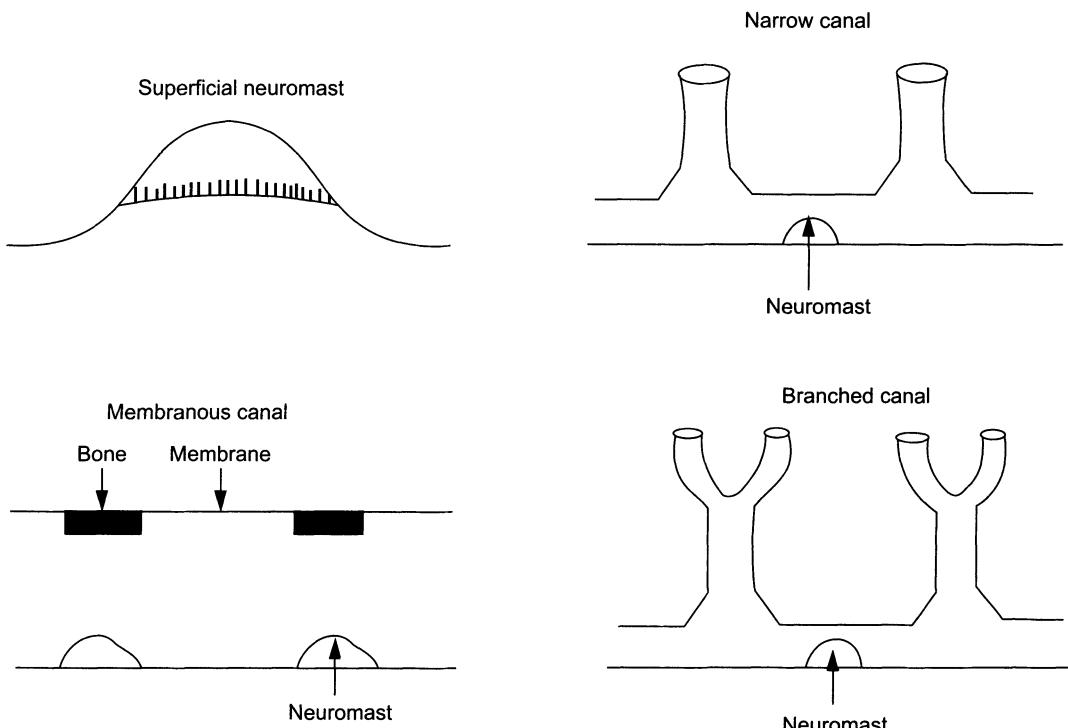
Fig. 3 Abstracted lateral line receptors and accessory structures.

includes head canals over (supraorbital) and under (infraorbital) the eyes and along the preopercle and mandible; head canals are usually connected to the trunk canal. In many species parts or all of the canals are missing. For example, the Anadyr River form of *Cottus cognatus* has a break in the infraorbital canal and two superficial neuromasts are in the same position as homologous canal neuromasts in the Lake Michigan form (Sideleva 1982). *Asprocottus herzensteini* and the other Abyssocottidae of Lake Baikal have no canals; superficial neuromasts on papillae replace the canal neuromasts (Fig. 4, Sideleva 1982). There is typically one trunk canal with associated neuromasts, but the canal can be split or multiple (Webb 1989) and sometimes very complex and anastomosing (Fig. 5, *Dictyosoma bugeri* and *Xiphister atropurporeus*, Stichaedae, Makushok 1961).

The typical "narrow canal" canal architecture consists of canals within skin and bone (or scales) with tubules that connect the canal to the water outside of the fish (Fig. 3). The neuromasts are found between the tubules. There are a few variations on this basic structure. In some fishes the tubules are branched and branching can increase as the fish grows (Fig. 4, *Aspicottus bison*, Cottidae, Neelev 1979). Fishes with "membranous canals" have greatly widened canals, with no tubule. A membrane that may have small pores covers the pore. (Fig. 3, *Comephorus dybowskii* in Fig. 4, Sideleva 1980, 1982). The pores are separated by bridge-like hard tissue that covers the neuromast (Fig. 3).
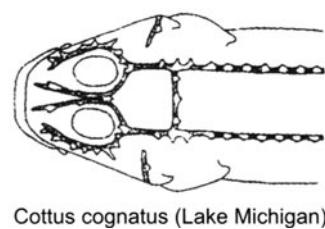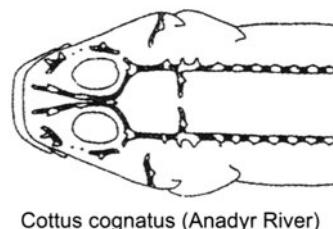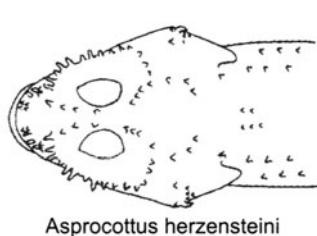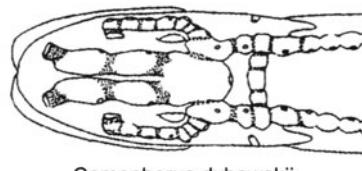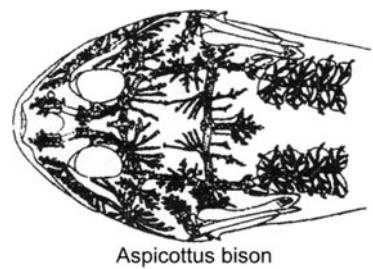
Fig. 4 The lateral line canals of several sculpins (Cottoidei). The two forms of *Cottus cognatus* have narrow, rigid canals; there is some canal loss in the Anadyr River form. *Asprocottus herzensteini* has no canals, the neuromasts are borne on the papillae. *Comephorus dybowskii* has wide, membranous canals. *Aspicottus bison* has branched canals. *Aspicottus* redrafted from Neelev (1979); all others redrafted from Sideleva (1982).

Batrachocottus baicalensis

Dictyosoma bugeri

Xiphister atropurpureus
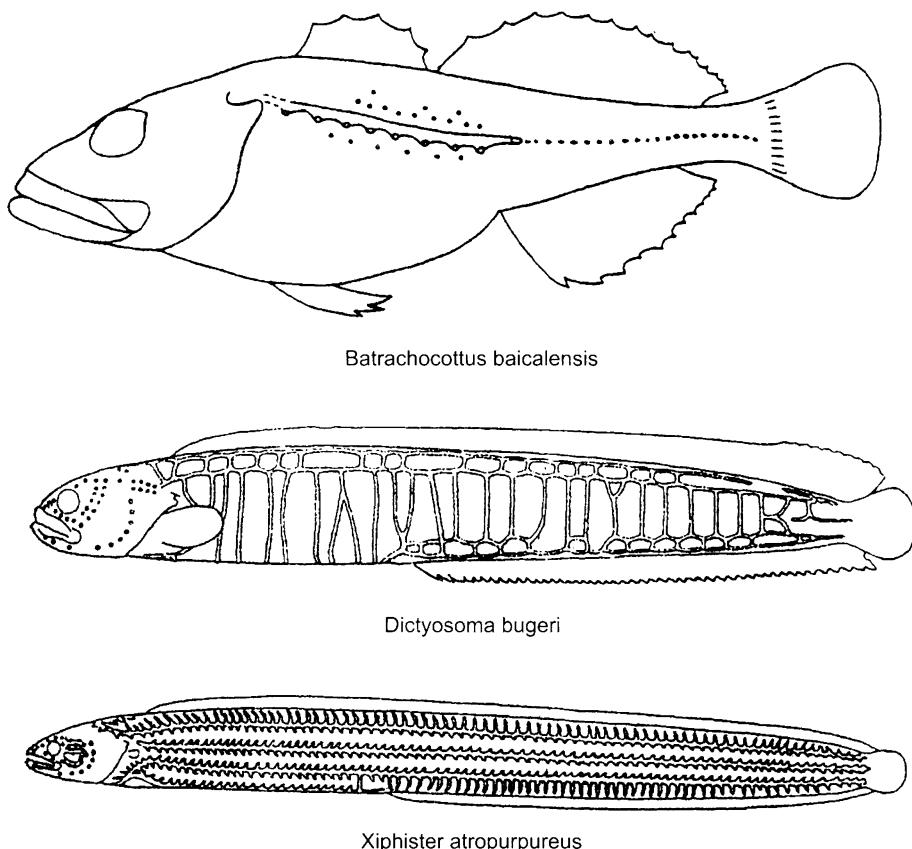
Fig. 5 Trunk lateral line canals. *Batrachocottus baicalensis* (Cottidae) has a trunk canal typical of many shallow water fishes, except the canal ends about mid-trunk and the posterior neuromasts are superficial. *Batrachocottus* redrafted from Sideleva 1982; *Dictyosoma* and *Xiphister* (both Stichaedae) redrafted from Makushok (1961).

Superficial neuromasts can have the sensory epithelium in pits, flush with the skin surface, projected on papillae as in *Asprocottus herzensteini* (Fig. 4) and especially certain ceratioid anglers (Marshall 1996). In *Asprocottus* and some other species it appears that the neuromasts may be retractable. Some neuromasts on papillae cannot be seen in a scanning electron microscope, but subsequent imbedding and sectioning shows a neuromast that appears to have been retracted. Whether this is an artifact of fixation or the fish can retract neuromasts is unresolved.

It is probably good procedure to assume that most lateral line systems are poorly described. Sometimes papillate superficial neuromasts have been confused with canal pores; McAllister (1968) counted 15-16 mandibular pores for the Baikal sculpin *Procottus jeittelesi* but the fish has only superficial neuromasts (Sideleva 1982). The misidentification may be because sculpin pores sometimes have extended tubules and the retraction of superficial neuromasts into

papillae leaves a blind pore. Superficial neuromasts can be difficult to locate because of the complexity of the epidermis in some fishes. Janssen et al. (1987) missed a pair of superficial neuromasts at the anterior of the mandible of the mottled sculpin; these were discovered while working with small individuals (Jones and Janssen 1992). Canals can be misidentified: Scott and Crossman (1973) described the skull of the freshwater drum (Aplodinotus grunniens, Sciaenidae) as having "a peculiarly strutted skull". The struts are the bony bridges that arch over the canal neuromasts of wide membranous head canals. The northern cavefish *Amblyopsis spelea* (Amblyopsidae) has a lateral line system with abundant superficial neuromasts and wide membranous canals, but the presence of the canals may have been noticed only by early workers (such as Putnam 1872); the canals were not mentioned by Poulson 1963. The organization of the cephalic lateral line is similar to that described for the pirate perch (*Aphrododerus sayanus*) (Moore and Burris 1956).

HYDROMECHANICAL AND LATERAL LINE ANATOMY

The signal at the neuromast is modified by the structures near it, hence, even for a simple signal, the spatial pattern of stimulus varies. A simple jet impacting the body othogonally spreads radially and parallel to the skin's surface. A deviation from orthogonal generates other flow patterns. Hassan (1993) has considered how a fish body alters the flow field from a pair of spheres oscillating 180° out of phase. Biological stimuli, being more complex spatially, are likely to produce very complex patterns of pressure and flow. It is likely that some fishes respond to only one or two parts of this complexity (such as where the maximum signal is) while others gather much more information and can distinguish things such as prey type. Lateral line canals respond to pressure differences along the array of canal receptors while superficial neuromasts respond to the flow itself (Hassan 1993). Whether expanses of fish surfaces are at all designed to capture some component of a signal is uncertain.

Superficial neuromasts and canal neuromasts differ in two ways. First, a superficial neuromast responds to any flow that is not orthogonal to its orientation axis. A canal restricts flow direction to the axis of the canal. Second, a canal acts as a high-pass filter, reducing the speed of flow for laminar and low frequency water movements outside the canal.

Hassan (1993) argued that the combined information from superficial neuromasts and canal neuromasts would allow a fish to gather much information about the source. The lateral line canal would register pressure distribution. Pairs of superficial neuromasts, oriented orthogonally, could gauge the direction of flow by comparing their outputs.

Narrow canals act as high pass filters by the action of friction on water moving in the canal. A fluid moving along a surface loses energy to the surface, forming a boundary layer with slower fluid nearer the surface. A tube has a relatively great surface area in relation to fluid volume, hence a boundary layer forms from the walls toward the center (Fig. 6). For a wider tube the boundary layer takes longer to fully form due to the greater distance to the center meaning more "layers" of water are accumulating. With non-oscillatory (DC) flow, or at low frequencies, flow in a tube is greatly impeded by the friction. As the frequency increases there is less time for the "layers" of water to accumulate along the canal wall, thus less energy has been lost to the wall,
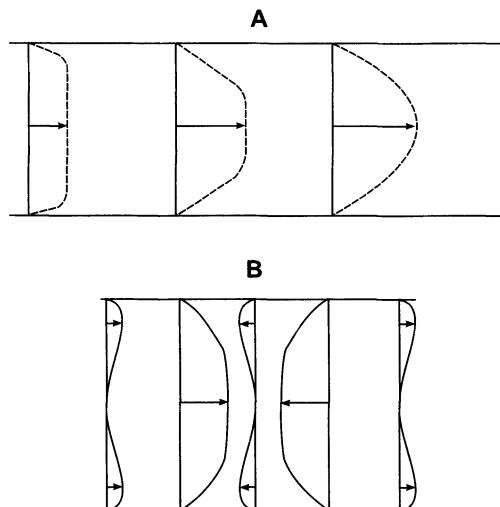
Fig. 6 Flows through tubes; arrows indicate direction of flow. Upper drawing shows DC flow as it enters a tube and the boundary layer develops. The fully formed boundary layer is on the right and is parabolic. Redrafted from Vogel (1994). Lower drawing shows AC flow in a tube as a time series from left to right. Redrafted from Schlicting (1968).

the boundary layer is less fully formed, and the flow is less impeded. Note in Fig. 6 how the velocity profile for maximum velocity for the alternating flow (AC, lower figure) resembles the velocity profile for the partially formed boundary layer for DC (upper figure). At a sufficiently great frequency the boundary layer is very thin, resembling that at the entrance to the DC tube, and the fluid acts "frictionless" (Schlicting 1968). As frequency increases from low frequency to high frequency the ratio between the velocity outside the canal and that inside the canal increases.

Boundary layers also affect superficial neuromasts; the difference is that the water acts "frictionless" at a lower frequency because there is relatively little surface area. The boundary layer thickness is increased by mucus on the skin (Daniel 1981) so calculations of boundary layer thickness based on water viscosity will be underestimates. As with canals, the boundary layer takes time to form. Engelmann et al (2000, 2002) reported a burst of presumed superficial neuromast afferent activity at the onset of a laminar flow current, followed by decreased but steady activity. The decrease in activity was probably due to the buildup of the boundary layer.

Denton and Gray (1988) offered a useful equation for approximating oscillatory flow in a canal:

$$V_{in}/V_{out} = (i\omega I_{out})/[(i\omega I_{in}) + R_{in})]$$

where i is the imaginary operator ($\odot$), ω is $2\pi*$frequency, I (a measure of inertia) and R (a measure of frictional resistance) are further defined mathematically and are functions of (for I) water density and canal cross-sectional area and (for R) dynamic viscosity, canal length, and canal crosssectional area. This equation makes explicit the major factors affecting flow inside a canal: V_{in} is inversely proportional to the sum of a component related to inertia (first component

in the right-hand denominator) and viscous forces (second component). A brief analysis of the equation demonstrates that as ω becomes large the velocity inside the canal approaches that outside the canal. This applies for a canal of uniform diameter. If there is a constriction at the neuromast then I_{in} is smaller than I_{out} and V_{in} exceeds V_{out} at sufficiently high frequencies (Fig. 7).

Denton and Gray (1988) derived their equation (and others for canals with narrow parts and membranous canals) for heuristic purposes and were able to demonstrate that it produced a reasonable match to empirical flow measurements. However they were careful to point out that their equation was a first approximation to the flow. For example, their equation would predict no canal flow for a DC current ($\omega = 0$) while Abdel-Latif et al. (1990) observed flow via the Bernoulli effect.

While the fluid in the canal tends to act as if there is minimal impedance at higher frequencies, the neural output eventually drops off because of the limitations of the sensory cells. Adjusting the empirical measurement of physiological response of a superficial neuromast for the flow predicted by Denton and Gray's (1988) equation produces an expected output very similar to that of a canal neuromast (Janssen 1996).

An alternative contrast presented by Kalmijn (1988) and Denton and Gray (1988) is that superficial neuromasts are "velocity sensitive" while canal neuromasts are "acceleration sensitive." It is the friction of the narrow canal that converts a neuromast from "velocity sensitive" to "acceleration sensitive." Superficial and canal neuromasts both respond approximately in proportion to the flow velocity along the cupula because the friction on the
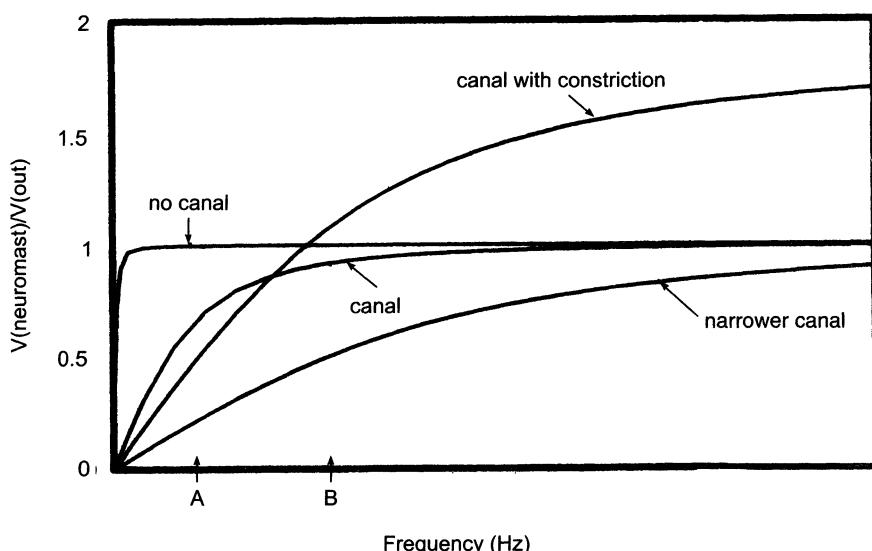
Fig. 7 Estimated flows past a superficial neuromast and in several canals. The abscissa is intentionally not quantified; the maximum frequency of a labeled abscissa might range from about 30 to 200 Hz. The points A and B indicate arbitrary low and high frequencies (compared in the text). From the equations of Denton and Gray (1988).

cupula increases with fluid speed. Because acceleration increases linearly with frequency (or ω) for a set maximum velocity during an oscillation, the hydromechanical and physiological responses are approximately linear with water acceleration outside the canal at lower frequencies.

It is easier to make comparisons between superficial receptors and canal receptors if stimulus measurements are made in the same units. Describing the canal receptors as "acceleration sensitive" and superficial neuromasts as "velocity sensitive" may be useful for physiological discrimination using an oscillating sphere, but it prevents a clear comparison of how the anatomy interacts with the signal. Fig. 7 shows some contrasts using Denton and Gray's equation. For example, while both the "canal" and "narrower" canal filter out low frequencies, the narrower canal is more effective, but at the cost of sensitivity. Keeping the measurement in units of velocity helps demonstrate the filtration aspect of canal physics. This aspect is lost when organs are classified as "velocity detectors" versus "acceleration detectors."

It may be difficult to physiologically distinguish canal neuromasts from superficial neuromasts. Coombs and Janssen (1990) used an acceleration sensitive/velocity sensitive measure for mottled sculpin afferent fibers and there was no bimodal distribution in the measure used. A large number of the fibers could not be clearly classified, probably due to lack of precision or a "noisy" response by fibers. Summation across hair cells in a neuromast probably increases the signal to noise ratio.

Schellart and Wubbles (1998) suggested that canal neuromasts, in comparison to superficial neuromasts, would be better able to respond to a stimulus onset or pulse because acceleration "leads" velocity. This is true for an ongoing sine wave because the magnitude of the acceleration is maximal when the velocity is zero. However, it is important to analyze the response of fluid in the lateral line canals from first principles. For example, for a transient jet pulsed parallel to the plane of the opening of a pore, the pressure (hence force) acting on the pore is negative and of maximum magnitude when the velocity is greatest due to the Bernoulli principle. There is no force acting on the canal fluid when the velocity is zero. Hence the fluid in the canal is still being accelerated while the fluid outside the canal has reached its maximum value. The superficial neuromast would receive maximal stimulation before the canal neuromast.

CANAL ARCHITECTURE, TUNING, NOISE REDUCTION AND SENSITIVITY

Narrow Canals

Dijkgraff (1963) argued that the function of lateral line canals was to reduce laminar and low frequency water movements that were likely to be noise. Acoustic noise in the sea, including infrasound is much greater at lower frequencies (Nichols 1981). Anatomical comparisons of species in a clade, are supportive of Dijkgraff's argument (Vischer 1989).

In a physiological test of Dijkgraff's (1963) argument, Engelmann et al. (2002, elaborating on Engelmann et al. 2000) compared evoked spike rate and phase coupling to an oscillating stimulus with and without a DC current for presumed superficial versus canal neuromasts. The

DC current masked responses by presumed superficial neuromasts more than for presumed canal neuromasts. While the neuromast types could not be identified as superficial versus canal with certainty, the two distinct classes nicely correspond to expectation.

The functional significance of the canal's ability to filter out laminar and low frequency noise is that a fish can detect higher frequency signals. For example, in Fig. 7, if there is a low frequency noise (turbulence) at frequency A and a prey signal at frequency B, the prey signal will be more masked by noise for the superficial neuromast than for the canal neuromasts. Note that each canal differs in its masking ability (See Janssen 1996).

Sensory systems need to be appropriately "tuned" to receive stimuli of biological importance but "tuned out" for irrelevant signals. There are compromises between being highly tuned (narrow band) and more broadly tuned. A sensory system narrowly tuned to prey may not function as well for detecting predators or for intraspecific signals.

Comparisons between closely related species that have diverged should offer the best evidence for or against tuning. Such a comparison has been made for presumptive canal neuromasts for several species of Antarctic fishes (Notothenioidea) including a mostly planktivorous fish that lives beneath the sea ice, two benthic species, and small and large piscivores (Montgomery et al 1994). Two benthic species had extremely variable tuning curves. It could be argued that different regions of the lateral line systems were tuned to different classes of signals (types of prey, predators, conspecifics), that different areas were subject to different types of noise, or that tuning curve measurements were very imprecise.

Variation in tuning may be more important than the average. Coombs and Montgomery (1992) reported a mean cutoff frequency (an estimate of the frequency above which the physiological response is decreasing) of 46 Hz for *Trematomus bernacchii*, but the standard deviation was 20.5 Hz, nearly half the mean. Assuming a normal distribution and precise measurement, approximately 65% of the fibers recorded would have cutoff frequencies between 25.5 and 66.5 Hz (+/– 1 standard deviation) and 95% of the fibers would be in a range of 5 to 87 Hz (+/– 2 standard deviations). These ranges may be too large because it is unlikely that the cutoff frequencies are measured with great precision. However, if the distribution is skewed the ranges increase.

The precision of characterizing fiber response is good enough to discern some species differences. For Antarctic notothenioid fishes, Montgomery et al. (1994) found statistically significant species differences in the variability (variance) of the frequency at which the response is 50% of maximum response (used as a high-cutoff frequency); some species were significantly more variable than others. Janssen (1996) statistically compared sensitivity data presented by Montgomery et al. (1994) and found *Trematomus bernacchii* was more sensitive than *T. pennellii*. The more sensitive species has wider canals, consistent with the expectation from Fig 7.

Branched Canals

In some species the tubules are branched and, as a consequence, there are two or more pores on either side of the neuromast (Figs. 3, 4, 5). The taxa are diverse (Coombs et al. 1988) and branching can increase as the fish grows.

I propose that the function of branching is to filter out small-scale hydrodynamic disturbances (Fig. 8). Consider the difference in flow past a neuromast situated between unbranched tubules versus a branched tubule with two pores. If the disturbance is spatially small so there is a pressure difference between the two pores of a branched tubule, then the flow is primarily between these two pores and there is little flow past the neuromast. If the disturbance is spatially larger so that both pores of a tubule have relatively high or low pressure, then there is greater flow past the neuromast.

Membranous Canals

Membranous canals are found in a variety of deep-living fishes as well as certain fishes found in turbid water. There are many taxa (Coombs et al. 1988).

Lateral lines with wide membranous canals are expected to be more sensitive than those with narrow, rigid canals. One reason for this is that there is more space for a larger neuromast with more hair cells. Denton and Gray (1988, 1989) offered a second reason: the membrane would have a resonant frequency and so amplify over some range of frequencies. They demonstrated a broadband resonance in the membrane of physical models (Denton and Gray 1988) and in the ruffe (*Gymnocephalus cernuus*, Percidae) (Denton and Gray 1989).

Because neuromasts are masses (the cupula) on springs (the kinocilia and stereocilia), they are expected to have resonance properties. Very large dome-shaped cupulae, such as are found in fishes with wide, membranous canals, have resonance frequencies (both predicted and
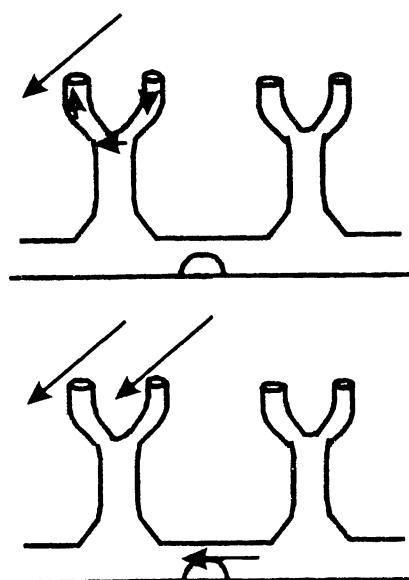
Fig. 8 Hypothesis about the function of branched tubules. Upper: With pressure induced by flow past one pore of a branch flow occurs mainly in the branch and there is less past the neuromast. Lower: with a broader signal, influencing both pores in a branch, there is flow past the neuromast.

measured) likely to be of behavioral importance (van Netten and Kroese 1987, van Netten 1991, van Netten and Marseveen 1994). Van Netten and Marseveen (1994) noted that the cupula resonance was strong enough to move the membranes over the canals.

A membrane has another effect, which is to convert the canal system into an accelerometer (Fig. 9), a point made to the author by S. Vogel and M. LaBarbera (Janssen 1997). The physics is different than that used to argue that narrow canals convert a neuromast into an accelerometer. As long as there is a constant pressure difference at two adjacent pores the conformation of the membranes does not change, so there is no water movement in the canal or past the neuromast. The membranes act as static plugs because the pressure difference is static. A change in pressure at one of the membranes causes fluid to flow in the canal. Hence a gliding fish with constant speed and no turbulence has a pressure distribution around its body that does not vary until the fish swims or there is an external stimulus such as a prey.

Some species have both an abundance of superficial neuromasts and wide-membranous canals. These include the pirate perch (Moore and Burris 1956), *Amblyopsis spelea* (pers. obs.), troutperch (*Percopsis omiscomaycus*, Percopsidae, pers. obs.), *Poromita capito* (Melamphaidae, Marshall 1996), *Melanonus zugmayeri* (Melanonidae, Marshall 1996), and Norway pout (*Trisopterus esmarki*, Gadidae, pers. obs.).

The papillate superficial neuromasts of the troutperch and pirate perch may be retractable. Scanning electron microscope preparations of papillae of these species often do not show a neuromast, but subsequent imbedding and sectioning reveal that the neuromast is mostly covered by tissue; a small pore leads to the neuromast. This may be an artifact of preparation, but it may also be that the fish can retract and protract neuromasts. Hence these fishes could be cruise predators with the superficial neuromasts retracted or ambush predators with the superficial neuromasts protracted.
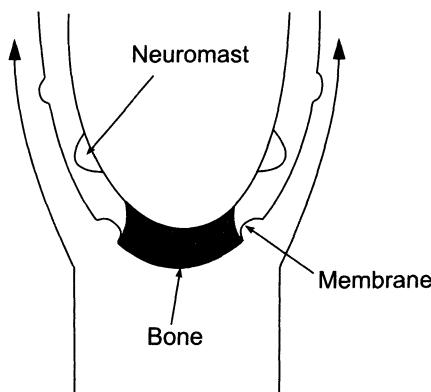
Fig. 9 Hypothesis about the function of membranous canals. The bone represents the snout or mandible tip and only two membrane-covered pores are shown on each side. When the fish glides (no thrust) there is laminar flow past the fish, high pressure at the anterior pores, and low pressure (due to the Bernoulli effect) at the posterior pores where the fish is widest. Because of the membranes there is no flow inside the canal so laminar flow noise is eliminated. A hydrodynamic disturbance causes membranes to move and generates flow inside the canal.

FUNCTIONAL MORPHOLOGY AND ECOLOGICAL CORRELATES

A "typical" lateral line system is generally considered to have narrow canals on the head and trunk, several lines of superficial neuromasts on the head and other superficial neuromasts along the trunk canal. This perception of "typical" is based on the regularly encountered fresh-water and coastal marine fishes. Most vertebrates (by number) are deep-sea dwellers of an es-sentially lightless habitat, however, and the "typical" lateral line is not generally found in these fishes. Fishes with an abundance of superficial neuromasts or wide membranous canals are rela-tively abundant.

Fish with abundant superficial neuromasts or wide, membranous canals are generally asso-ciated with quiet environments and/or are less active species (Dijkgraff 1963, Marshall 1971, 1979). There are exceptions, however. The gobies (Gobiidae) have most or all of their canal neuromasts replaced with superficial neuromasts (Miller 1986) and are frequently found in streams on oceanic islands. It may be that, because many gobies are tolerant of freshwater, they occupy vacant freshwater niches without having lateral lines well adapted to streams. Most cavefishes have an abundance of superficial neuromasts and more species of gobies have adapted to freshwater caves than any other fish family (Parzefall 1986). It may be that gobies are pre-adapted to live in a cave environment because of their lateral line system (Jude et al 1995).

Many fishes with abundant superficial neuromasts have body shapes inconsistent with active swimming. These include the deep-sea anglers (Ceratiodei) with their globular bodies and the elongate gulper eels (Eurypharyngidae, Marshall 1979).

Fishes with wide, membranous canals can be fairly active. While searching for stationary prey in the dark, ruffe (wide, membranous canals) swim faster, but less erratically than yellow perch (*Perca flavescens*, Percidae; narrow canals, Janssen 1997). Ruffe detect prey during glides following pectoral fin flaps. Northern cavefish also use a pectoral stroke and glide (Poulson 1963). This fish has both wide membranous canals (pers. obs) and the abundant superficial neuromasts described by Poulson. An interesting study would be whether the northern cavefish uses the membranous canal neuromasts for prey detection while gliding, but can also be an ambush predator and switch to use of the superficial neuromasts. The deep benthopelagic orange roughy (*Hoplostethus atlanticus*, Trachichthyidae), with a wide membranous canal simi-lar to that of *Anoplogaster* (Anoplogasteridae) described by Denton and Gray (1988), swims with labriform pectoral rowing (pers. obs. Johnson Sea-Link submersible video).

Body undulation can be restricted to the tail to minimize head motion. The mesopelagic barracudina *Notolepis rissoi* hovers (head up) or swims (many angles) with most of its body rigid, undulation being limited to near the caudal fin (Janssen et al. 1992). The fish has membranous head lateral line canals and the trunk canal is also membranous, ending where the red muscle used for hovering begins.

Some fishes with wide membranous canals appear to actively ascend, then glide passively. *Comephorus dybowskii* (Comephoridae), a mesopelagic sculpin from Baikal, Russia that has tiny eyes and a wide membranous lateral line canal (Fig. 4), extends its large pectoral fins (45% of

the body length) as "wings" for a diagonally descending glide (Sideleva et al 1993). The fins are folded along the body when the fish uses caudal propulsion to ascend. Its body density is 1.04, denser than its nearly neutral congener *C. baicalensis* that has large, almost tubular eyes (Pankhurst et al.1994). *Bathyclupea* (Bathyclupeidae), a deep benthopelagic species, sinks head down until it's snout touches the bottom, then ascends, head still down and tail-first, rowing backwards with its pectoral fins (pers. obs., Johnson Sea Link Submersible). While observations on *Bathyclupea* were made with submersible lights, the several individuals observed each had "chalky" snouts prior to hitting the bottom suggesting that bottom contact was frequent. Bioacoustic traces of Norway pout, which also has membranous canals, shows a pattern of swimming up and sinking through layers of krill (*Euphausia*) (S. Kaartveldt, University of Oslo, pers comm).

While a variety of fishes with wide membranous canals have swimming patterns that would minimize changes in membrane shape, these swimming patterns are also found in fish with narrow rigid canals. The alewife (*Alosa pseudoharengus*, Clupeidae) detects prey and oscillating spheres in the dark during glide phase that alternates with a tail beat. Elongate mesopelagic snake mackerels (*Nesiarchus, Benthodesmus*, Gempylidae), and *Serrivomer* (Serrivomeridae, pers. obs. Johnson Sea Link submersible) have a head-up swimming pattern with undulation restricted to the tail very similar to that of *Notolepis*, yet have narrow rigid canals. The lack of head movement probably enhances both lateral line and visual function in the dark mesopelagic.

It may be that some membranous canals function as barriers to accumulation of particulates. Moore (1956) found dirt in the canals of the orangespotted sunfish (*Lepomis humilis*), a species of turbid streams. Fange et al (1972) suggested that the membranes served as a mud barrier in macrourids, which are benthic and benthopelagic. The burbot (*Lota lota*, Gadidae) and the deepwater sculpin (*Myoxocephalus thompsoni*, Cottidae), have wide membranous canals. Both dig depressions in silt. The deep habitat of both in the Great Lakes has currents (laminar) typically of about 10 cm sec^{-1} but can be as low as nil and as high as 20 cm sec^{-1}. Hence the membranous canals could function to remove laminar flow noise as proposed by Janssen (1997). It is unlikely that the membranous canals in pelagic fishes function to seal the lateral line canals from mud.

Fish with wide membranous canals can be very large and trophically diverse. The drums (Sciaenidae) include species ranging from about 25 cm to 150 cm. Larger species such as the redfish (*Sciaenops ocellatus*) tend to be piscivorous and smaller species are plantivorous.

The ecological function of branched canals is unclear and may be diverse. The fishes that have them are in many clades (Coombs et al. 1988) and are ecologically diverse. For example, the gulf herring (*Brevoortia patronus*) is a pelagic, schooling ram filter feeder that consumes primarily phytoplankton (see Gunter and Demoran (1961) for description of lateral line) and the sculpin *Aspicottus bison* (Fig 4) is benthic and feeds on invertebrates.

USE OF THE LATERAL LINE SYSTEM IN BEHAVIOR

Determining the Involvement of the Lateral Line

Use of mechanosense (hearing and lateral line) is presumed when the fish are either blinded or experiments are run in the dark, often using infrared light, and the stimulus has no chemical or electrical component. Because no behavioral experiments have eliminated the hearing sense it cannot be argued that the lateral line is sufficient for any particular behavior, only that it is involved. For example, the anuran *Xenopus laevis*, makes accurate orientation movements to surface waves. The frogs respond with reduced accuracy when the lateral line is eliminated. The neural mechanism of localization may involve both hearing and lateral line (Elepfandt 1982). Some species, notably herrings (Clupeidae), have physical coupling between the lateral line and auditory system such that displacements in the canal fluid affect the ear.

Means of eliminating the lateral line or components of it include pharmacological treatment, physical blocking (paste:Hoekstra and Janssen 1985; threads: Abdel-Latif et al. 1990, Janssen 1996, and Janssen and Corcoran,1998), cutting a lateral line nerve (Sutterlin and Waddy 1975, New et al. 2001), cauterizing superficial neuromasts (Janssen 1996), and scraping (Montgomery et al. 1997). Scraping a fish to remove the cupulae of superficial neuromasts may release an alarm pheromone in some fishes, especially ostariophysans.

Pharmacological blocking agents include streptomycin (Kaus 1987), cobalt with low calcium concentration (Karlsen and Sand 1987) and gentamicin, which apparently blocks only canal receptors (Song et al. 1995).

Unwanted behavioral side effects or mortality can occur with cobalt (Janssen 2000) or streptomycin (Kaus 1987). Juvenile mottled sculpin die before the Karlsen and Sand (1987) cobalt treatment is finished (Carvan and Janssen, unpublished). Janssen (2002) reported altered swimming behavior and mucus production, both typical of heavy metal stress, at concentrations used in rheotaxis studies (Montgomery et al. 1997, Baker and Montgomery 1999).

Cobalt acts on the lateral line via competitive inhibition with calcium (Karlsen and Sand 1987). However, because calcium is used in osmoregulation and cobalt toxicity is increased when Ca^{2+} is low (Diamond et al. 1992), fish may be stressed. Ringer (1883) reported the toxic effects of distilled water, so when fish are treated with $CoCl_2$ in otherwise ion-free water (e.g. Abboud and Coombs 2000, New et al. 2001) it is unclear whether behavioral changes are due to osmotic stress, loss of neuron function due to loss of sodium, potassium and calcium, or lateral line inhibition.

Recovery of sensitivity after cobalt treatment probably varies with species, temperature, and calcium content. Karlsen and Sand (1987) reported measurable recovery within 3 hr and substantial recovery at 2 hours or less occurs in blind Mexican cavefish (*Astyanax hubbsi*, Characidae, Hassan et al. 1992). Coombs and Conley (1997), New et al. (2001), and Abboud and Coombs (2000) removed their fish from cobalt treatment in ion-free water and assumed the lateral line was inhibited for several days. It is best to apply cobalt chronically, as per Liang et al. (1998) did, and extend the treatment beyond the experiment to check for evidence of toxic stress (Janssen 2000).

Speedometers and Orientation in Currents

Swimming is a potential source of stimulation to the lateral line; the clearest evidence that it is used as a speedometer is the work of Hassan et al (1992). They showed that inhibition of the lateral line system by treatment with low calcium concentration or inhibitory cobalt increased swimming speeds of blind Mexican cavefish. Presumably reduced lateral line activity is perceived as slower than actual swimming speed.

Water motions generated by swimming are a source of self-generated noise (Denton and Gray 1993). Caudal propulsion in most fishes produces a side to side head movement, which generates oscillations in flow and pressure, especially at the snout (Dubois et al. 1974). In some cases these signals are noise, but the signals are also a possible source of information to the fish. Rowe et al. (1993) suggested that appropriate swimming technique might minimize this self-generated hydrodynamic noise.

The difference in physiological response of superficial ("velocity sensitive") versus canal neuromasts makes the superficial neuromast an appealing class of receptors to mediate rheo-taxis. This was argued by Montgomery et al. (1997) and Baker and Montgomery (1999), but, their higher than recommended cobalt concentrations causes aberrant swimming (Janssen 2000).

The focus on canal neuromasts as being acceleration receptors has probably resulted in their not being considered as a rheotactic monitor. However, Abdel-Latif et al. (1990) found that dye injected into a model lateral line canal on a model blind Mexican cavefish streamed from the canal when the "fish" was in flowing water. The flow is due to the Bernoulli effect and because the drop in pressure is proportional to the square of the water velocity, the Bernoulli generated flow will increase dramatically with fish speed.

Brook trout (*Salvelinus fontinalis*, Salmonidae) orient behind objects in streams. They can do this non-visually but the ability is diminished by cutting of the posterior lateral line nerve (Sutterlin and Waddy 1975).

Schooling

Schools in many fish species break up at night, but at least one fish (saithe, *Pollachius virens*, Gadidae), when fitted with eye covers, can maintain schooling (Pitcher et al. 1976). Sectioning of the trunk lateral line nerve eliminates this ability. Herrings are also schooling fishes, but they lack a canal along the length of the trunk (there are superficial neuromasts along the trunk however, (Janssen et al. 1995)). Denton and Gray (1983) and Gray (1984) suggest a mechanism by which the connection between the lateral line and ear in herrings would provide information about the distance to a neighbor in a school.

Prey Detection

Predation is useful for probing the abilities of the lateral line system because the behavior is unconditioned. Fish respond well to food rewards so can be trained for psychophysical studies.

The relative importance of canal vs. superficial neuromasts for feeding probably varies between species. Differential ablation indicates that canal organs are more important in *Trematomus bernacchii* (Janssen 1996) and mottled sculpin (Coombs et al. 2001). Janssen & Corcoran (1998) found diminished ability of mottled sculpin to determine distance to a source presented along the fish's flank on the side where the trunk canal was blocked by a thread.

However, other fishes may rely entirely or primarily on superficial neuromasts for feeding. *Procottus jeittelesi* (Cottidae, similar to *Asprocottus*, Fig. 4) from Baikal have only superficial neuromasts and readily respond to hydromechanical stimuli (Janssen et al. 1990a). The canals do not form in this species, probably a consequence of paedomorphosis, so many of the neuromasts should retain canal neuromast "wiring." Abdel-Latif et al (1990) found that the canal receptors were not needed for feeding in the blind Mexican cavefish. Soles (Soleidae) have an abundance of superficial neuromasts arrayed between papillae on their abocular side that presumably are used for detection of their buried prey (Applebaum and Schemmel 1983, Harvey et al 1992).

The degree to which a fish uses various regions of the lateral line for feeding may vary. For example, two benthivorous Antarctic fishes, *Trematomus bernacchii* and *T. pennelli* (Nototheniidae) respond to hydromechanical stimuli at both the head and trunk. In contrast, the benthic planktivore *T. nicolai* and a planktivore that lives under the sea ice, *Pagothenia borchgrevinki*, respond only to stimuli near the head (Janssen 1996). Stimuli to the trunk are ignored or elicit an escape response. A possible reason for the difference is that the benthivores can turn to reposition without causing prey to be swept away by the maneuver.

Predation can be broken into a sequence beginning with a decision to search (or be alert) to prey, followed by detection and localization of the prey, and ending with a decision on whether to attempt capture. All aspects are likely to generate selection pressures on the lateral line.

Search behavior is expected to impact sensitivity to prey because of self-generated noise. Search behaviors range from inactive, ambush predators, to active, cruising predators. Saltatory searchers combine elements of ambush and cruising by frequently moving from one stationary search position to a new one. Prey are detected only from the search positions and the pause time (time until the fish quits a search position because no acceptable prey was detected) varies with species and experience. Visual examples include the bird genus *Turdus* (American robin and European blackbird, Eiserer 1980, Smith 1974) and sunfishes (Centrarchidae, see Janssen 1982, Ehlinger and Wilson 1988, and O'Brien et al. 1989).

Fish movement creates self-generated hydrodynamic noise of low frequency (Bleckmann et al. 1991) that can mask the prey's signal. Mottled sculpin (lateral line similar to *Cottus cognatus* from Lake Michigan, Fig. 4), search in the dark, or when blinded, in a saltatory pattern. During the stationary phase the opercle is held open. This stops respiratory currents and also rounds and expands the head outline, perhaps increasing the receptive area. For artificial signals, constant flow (DC) or pulsed flow jets, the time spent in a search position is less for more detectable signals (Janssen et al. 1990b).

A variety of fishes become quiescent at night; this reduces hydromechanical noise so they can both be cryptic and better detect prey. Yellow perch are saltatory when visually searching for

Daphnia, but become ambush predators when feeding on *Daphnia* in the dark (Janssen 1997). However, in the dark, yellow perch are active searchers for burrowing mayflies, a prey that does not move from its burrow.

Because canal architecture is related to noise reduction, it is expected that fishes with wider canals will be less active, but be more sensitive, than fishes with narrower canals. This applies for two shallow water sculpins of Lake Baikal (Janssen et al. 1999). The more sensitive species, *Batrachocottus baicalensis* (Fig 5) has wide non-membranous canals and is an ambush predator while the less sensitive *Paracottus kneri,* with narrower canals, is a saltatory searcher. The canal arrangement of both species is similar to *Cottus cognatus* from Lake Michigan (Fig. 4). Both species have canals constricted in the vicinity of the neuromasts, which should amplify the prey's signal. Only *Paracottus* would feed in a hydrodynamically noisy system, an artificial stream. The diet of *Paracottus* is more diverse than that of *Batrachocottus,* perhaps because *Paracottus* moves through more microhabitats during its saltatory search (Janssen et al. 1999).

The endemic Baikal benthic sculpins include two non-endemic species common in the surrounding streams and 20+ endemic species of which at least four (*Batrachocottus baicalensis* and congeners) have very wide canals and the rest have only superficial neuromasts (similar to *Asprocottus* in Fig. 4). It is likely that the many tributary streams and turbulent draining Angara River imprison these endemic sculpins to Baikal because their lateral lines will not function well in the hydrodynamic noise (Janssen et al. 1999). *Paracottus,* with its narrower canals, is abundant in the neighboring streams.

Fishes with an abundance of superficial neuromasts are expected to be more sensitive to prey than fishes with fewer neuromasts. This is true for the round goby (*Neogobius melanostomus,* Gobiidae) which lacks most canals and superficial neuromasts have proliferated in a pattern typical for gobies (Miller 1986, Marshall 1986). Its response distance to *Daphnia* is greater than that for mottled sculpin (Jude et al. 1995).

It is expected that fishes with wide, membranous canals would be more sensitive and possibly more active (because of laminar flow noise reduction via the membranes) than those with narrow canals. This is the case with ruffe (wide membranous canals) vs. yellow perch (narrow canals, Janssen 1997) feeding on stationary ventilating mayflies or *Daphnia.* The ruffe detected its prey during the glide phase of its swimming while yellow perch were either stationary (*Daphnia*) or moved more erratically (mayflies). The combination of increased speed and sensitivity allows ruffe to feed at about twice the rate of yellow perch on mayflies in the dark.

Gliding probably reduces noise for any lateral line system. Detection of prey during the gliding phase is characteristic of alewives (*Alosa pseudoharengus,* Clupeidae), a species with branched head canals (Janssen et al. 1995). The alewife uses caudal propulsion, a tail beat or two, before each glide. In contrast, the ruffe uses pectoral flapping and the body is fairly rigid except that it curves its body for turns. Janssen et al. (1995) did not measure response distance for alewives feeding on *Daphnia;* the distances appeared to be short and were difficult to measure. They were almost certainly shorter than response distances for ruffe based on results for yellow perch, which had a shorter response distance than ruffe.

Prey Position Determination

Having neuromasts arrayed around a fish's body extends its receptive field to form a "halo" around the body and also creates the possibility that, at least some species, can compare inputs across the array to track movement or estimate distance. The array is usually more elaborate on the head, which suggests a computation ability that is usually lacking on the trunk. The elaborate trunk lateral line canals in stichaeids (Fig. 5) suggests that these species do more sophisticated spatial analyses of signals near the trunk that species with simpler lines.

A useful skill would be to track the movements, via the "hydrodynamic footprints" of other organisms. This has been suggested for a catfish (*Siluris glanis*) but the catfish could have tracked prey via a chemical trail or a combination of a chemical and hydrodynamic footprints (Pohlmann et al. 2001). Similar tracking to locate mates has been documented in copepods (Doall et al. 1998).

A spatial array of lateral line receptors has the potential for calculating the position of a source. This physiological potential was demonstrated by Gray and Best (1989) for the ruffe. Small changes in the position of a stimulus produced large changes in the spatial pattern of neuromast activity. Blinded mottled sculpin are able to determine distance from the trunk to a sphere making a single pulse oscillation (Janssen and Corcoran 1998). Distance determination apparently was based on the pattern of stimulated neuromasts, not on the attenuation of the signal with distance. Stimulus amplitude compensated for distance and random amplitude was added so that the only consistent change with greater distance was the decrease in curvature of the radiating pulse.

A measurement of precision should have biological meaning. The fish's criterion for success is capture of the prey. Hoekstra and Janssen (1986) empirically defined a "strike zone" for mottled sculpin feeding on *Daphnia*. A *Daphnia* within the strike zone would be sucked in without the fish repositioning. Hoekstra and Janssen determined how well the fish could reposition its head if the *Daphnia* was initially along its trunk, hence outside of the strike zone. The fish tended to reposition so that the target was in the strike zone rather than at the snout. This is appropriate because mottled sculpin have tiny teeth typical of suction feeders as opposed to graspers. Janssen and Corcoran's (1998) work on distance determination suggests that, even when rewarded only for contact of an artificial stimulus initially positioned off the trunk, the mottled sculpin tends to reposition so the stimulus is in front of the mouth, in the strike zone. Misses may not have been biologically meaningful misses because the artificial prey could not be sucked in.

A stimulus can cause strikes that are off-target. Janssen et al. (1990) reported that mottled sculpin strikes at a water jet were more likely to be off-target for a DC jet in contrast to AC jets of similar maximum flow. This may be because a DC jet has less information than an AC jet; the AC jet would better stimulate the canal neuromasts and so both canal and superficial neuromasts would be involved in the distance "calculation", consistent with Hassan's (1993) argument. Janssen and Corcoran (1993) reported that the strike direction in response to water jet onset in two sunfishes (Centrarchidae) was determined by where the jet impacted the fish

rather than the jet's vector. For example, a jet impacting the tip of the snout elicited a strike forward even if the jet nozzle was to the side. Escaping crustacea probably generate similar jets. It would be interesting to know whether the sunfishes better localize a more complex stimulus (i.e. with an AC component).

It has been argued by Coombs and Conley (1997) and Coombs (1999) that mottled sculpin are poor at determining source location for oscillating spheres in front of the snout as opposed to the side of the head. Using a criterion that non-contact is a miss, most of the misses noted by Coombs and Conley were +/− 20° from in front of the snout. With suction feeding the fish does not need to be as near to the stimulus. From the fish's perspective, the prey probably escaped.

Most work on prey localization in mottled sculpin has focused on the horizontal response. This is ecologically appropriate because their prey is benthic and epibenthic (Hoekstra and Janssen 1985). Hoekstra and Janssen noted that the fish did respond to stimuli above the head by arching the vertebral column, retracting the anal fin, and propping itself up on its pelvic fin. Abboud and Coombs (2000) quantified the response but did not measure the precision, e.g. alignment to the stimulus.

Partial ablation of the lateral line suggests that localizing a source depends on the array of receptors. Hoekstra and Janssen (1985) covered areas of mottled sculpin lateral line with a gel. Partial coverings, e.g. covering the top of the head, eliminated body arching and fin adjustments used to orient to stimuli above the bottom. Janssen (1996) physically blocked the head canals of *Trematomus bernacchii* with nylon threads. Blocking on one side (left or right) caused off-target strikes biased to the intact side. Blocking of all head canals eliminated strikes to hydromechanical stimuli. In contrast, unilateral blocking of the trunk canal in mottled sculpin reduces accuracy to stimuli on the blocked side, but does not eliminate the response (Janssen and Corcoran 1998).

Coombs (1999) argued that mottled sculpin are more likely to make "false alarm" attacks in a forward versus a lateral direction. A "false alarm" is a situation in which the fish's nervous system initiates a response to a signal when there is no signal. Coombs' criterion for a false alarm was a motion (within set criteria) when no stimulus was generated. This criterion generates many errors because it assumes mottled sculpin are ambush predators and move only when they "think" there is a stimulus. As saltatory searchers, mottled sculpin move both when a prey is detected and to abandon a position at which no prey were detected (Janssen et al. 1990). Movement is usually forward for biomechanical reasons and usually after about 1-2 sec at a position. An unambiguous criterion for a false alarm would be a strike when there is no stimulus; such false alarms have not been reported.

Blinded muskellunge (*Esox masquinongy*, Esocidae) appear to have great precision when locating fathead minnows (*Pimephales promelas*, Cyprinidae; New et al. 2001). The mean average angular deviation from the long axis of the muskellunge body was 7.4° (S.E. = 0.7) indicating prey were mostly directly in front of the predator. The restricted strike zone was not due to limitations in the biomechanics of the strike; muskellunge with eyes had a larger mean angular deviation of 18.4° (S.E. = 2.3). Both visual and blinded fish had similar success rates for capture, about 73%, but strike distance was shorter for blinded fish.

The muskellunge and mottled sculpin work provide some evidence for a "lateral line fovea." The mottled sculpin repositions itself to place its prey in a strike zone that is spatially restricted (Hoekstra and Janssen 1986), much as a human eye moves to place an important item on the fovea. For the muskellunge the lateral line fovea is perhaps more restricted than the visual fovea, in that the attack angle is broader when vision is involved. However, muskellunge can move their eyes so the possible target area on the retina is probably more restricted than when measured relative to the body axis. Muskellunge and mottled sculpin have similar head lateral lines, but the muskellunge head is elongate versus round in a mottled sculpin in search position. Because of the elongate snout few neuromasts are near a prey near the body axis. This may mean that localization requires relatively few neuromasts.

Other evidence for a lateral line fovea comes from *Chromis viridis* (Pomacentridae) feeding on evasive calanoid copepods (Coughlin and Strickler 1989). Proper alignment of the fish's mouth and prey are essential for the fish to avoid triggering an escape. The small capture current may actually trick the prey into swimming into the mouth. The fish is much less able to properly align (acquire) its target when the signal is purely visual, a hologram.

Surface Feeding

The most elegant experiments on the use of the fish lateral line are in regard to surface feeding fishes. Several species of neustonic fishes respond to surface waves generated by struggling animals. Fishes are able to judge both direction and distance to the target. Part of what has made this field so productive is that the stimulus, capillary waves, are relatively simple and easy to quantify/characterize. This work is well reviewed by Bleckmann (1994).

Buried Prey

Animals burrowing in or traversing on substrate produce a variety of substrate vibrations. Use of these signals has been studied for a variety of terrestrial animals including reptiles, amphibians, and arthropods. Janssen (1990) showed that substrate-borne signals could also be used by mottled sculpin. Live prey included a burrowing oligochaete typical of its diet, and *Nereis*, a marine polychaete (not part of its diet). Fish approached only when the prey moved. Fish quickly responded to an oscillating sphere in the substrate with an orientation response, placing its head to the substrate, and a saltatory approach with the head to the substrate. The approach culminated with the fish plunging its head into the substrate to bite the sphere. A sphere above the substrate was not detected unless the fish was very close. There is probably some interaction between the ear and the lateral line in this behavior; treatment with cobalt or streptomycin eliminates the saltatory approach, but the fish quickly responds to stimulus onset by placing its head to the substrate.

Response to substrate vibration would be expected from a variety of benthic fishes. Blinded southern flounder (*Paralichthys lethostigma*, Bothidae) respond with cessation of breathing to a dropped weight striking a sand bottom about 2 m away. If the weight instead strikes sand in a container suspended above the bottom the fish continues its breathing (pers. obs.). Soles

(Soleidae), which feed more on buried prey than bothids and pleuronectids and have an abundance of superficial neuromasts on their abocular side, would be likely candidates for the use of substrate vibrations for finding prey. Soles make less use of light when feeding than flounders (Batty and Hoyt 1995).

Ontogeny of Feeding

It is likely that the lateral line is very important in early life stages because visual acuity and light gathering and resolution in small eyes is poor (Pankhurst 1994, Higgs and Fuiman 1996). Mechanoreception is common in small invertebrates such as planktonic crustacea and insects (Pastorok 1981, Yen and Strickler 1996), so it may be a more useful sense than vision for small animals.

The mottled sculpin undergoes an ontogenetic change in lateral line anatomy, behavior, and sensitivity to *Artemia* metanauplei. The neuromasts initially are all superficial. Canal formation begins at the head; as these form the response distance to *Artemia* decreases. While the head canals are forming, the trunk canal has yet to form and the response distance stays constant for prey along the trunk. The results are consistent with the prediction of a reduction in sensitivity for a neuromast changing from superficial to enclosed inside a canal.

It is peculiar that sensitivity decreases during development, even if consistent with what would be expected from enclosing neuromasts inside canals. Jones and Janssen (1992) argued that canal formation was a potential sensory bottleneck in which sensitivity decreased temporarily until canal architecture was complete. Alternatively, as the head canals form, the fish become more active searchers, changing from an ambush to saltatory search. Comparing the five youngest fry (no lateral line canals formed) from Jones and Janssen (1992) versus the five oldest fry (head canals mostly complete), the pause time is longer for the youngest fry (means: 86 sec. vs 13.1 sec, nested ANOVA with pause times nested within individuals, F = 24.7; 2, 8 df; P < 0.001. Unpublished data from Jones and Janssen 1992). This reanalysis suggests that, once canals are formed, the fish are less constrained by the noise generated by their own search movements and become more active searchers.

Canal formation, or perhaps maturation of the architecture, may be important in the feeding of some fishes. Radischcheva et al. (1991) found that pike (*Esox lucius*) did not feed in the dark until they reached 22-30 mm, well after they had begun visual feeding.

Predator Detection and Avoidance

Work on predator detection has focused primarily on larval fish. This is very appropriate because of the intense predation pressure on early life stages. Blaxter and Fuiman (1990) found that canal formation was related to escape abilities in herring (*Clupea harengus*). There was no impairment by streptomycin on herring attacked by a natural predator until the head canals formed. These canals have a connection to the inner ear, indicating that the mechanism may involve the ear. However, the ear of herrings has a connection to the swim bladder that is expected to enhance hearing; the inflation of the auditory bulla has no effect on escape ability

in another herring, the menhaden (*Brevoortia tyrannus*, Higgs and Fuiman 1996). The lateral line is also involved in escape by Atlantic croaker larvae (*Micropogonias undulatus*, Poling and Fuiman 1995).

Gregory (1993) reported that young chinook salmon (*Onchorhynchus tshawytscha*) escaped into turbid water when they detected a predator. This strategy could put the prey at an advantage if the predator attempted to follow. The predator's larger size would likely produce a large hydromechanical stimulus.

The thrust and glide swimming of ruffe and alewife when searching for food in the dark would create a punctuated hydrodynamic trail that may be more difficult for a predator to track. This might be particularly effective if the fish frequently turned in a protean manner.

Social and Reproductive Interactions

A common agonistic display in fishes is "tail beating" in which one fish produces exaggerated tail oscillations directed at an opponent. There is obviously a hydrodynamic signal. Dijkgraff (1967) reported tail beating in spawning blinded fish and I have seen tail beating in pairs of blinded *Trematomus bernacchii*.

Whang and Janssen (1994) reported that vibrations produced by mottled sculpin males in the presence of females traveled through the substrate. The sounds were produced during head nods and the fish also slapped their heads on the substrate. Head slapping also occurs in the round goby (*Neogobius melanostomus*, Gobiidae, K. Wolfe, pers. comm,). In the field the sound is much clearer using a geophone than a hydrophone. It is not clear whether the lateral line is involved in detection of the vibrations, but Janssen (1990) reported that the lateral line was used in approaching artificially generated substrate vibrations. Mottled sculpins commonly spawn in rocky riffles that produce considerable noise for humans. Whang and Janssen (1994) found little noise in the substrate of a natural spawning riffle so the substrate provides a quiet "channel" for communication.

THE LATERAL LINE AND OTHER SENSORY SYSTEMS

It is unlikely that sensory systems "compete" with each other for primacy. Cooperation between sensory systems ought to benefit the fish. For example, wake tracking in catfish (Pohlmann et al. 2001) may depend on integrating chemical and hydrodynamic stimuli. Investment in one sensory system may decrease the potential for investment into others. A very large eye occupies much of a fish's head that might otherwise be used for the lateral line, touch, or taste. The space for a brain may also be limiting forcing neural compromises (Poulson 1963).

Adaptations for one sense may constrain other senses. Pankhurst and Montgomery (1989) showed a correlation between eye position and the orientation of the mouth in Antarctic nototheniid fishes. Fishes with a mouth designed to capture prey above the fish tended to have eyes somewhat dorsal in position. Janssen (1996) found that lateral line structures, particularly the size of neuromasts and canal diameters suggested an orientation similar to that of the eye and mouth.

Janssen and Corcoran (1993) reported that a water jet onset could override a visual cue in determining feeding strike trajectory in two sunfishes (Centrarchidae). A jet aimed at the head elicits a strike from many fish species with intact eyes (including Nototheniidae (Janssen 1996), Centrarchidae, Cichlidae, Cottidae, Percidae, and Esocidae, pers. obs.). Janssen and Corcoran (1993) argued that their experiment did not demonstrate the primacy of the lateral line sense, but that a fish uses whatever sensory inputs are available. A prey might be detected first by vision as it escapes into loose material on the bottom. Because the prey is now visually cryptic, the predator may then best detect a respiratory current. It is likely that one sense is better at scanning for prey and one is better for positioning for a strike.

Twilight at dawn and dusk are times when predators are very active. This also a time when visual and hydrodynamic signals are both being used. Janssen et al. (1995) noted that the major nocturnal prey of alewives in the Laurentian Great Lakes, mysid "shrimps," migrate up at night to a depth that placed the mysids at the dark edge of fish visual competence. The mysids are at a light level at which they are probably not visible. Even if cloaked in darkness, the alewife can use its lateral line to feed in the dark.

PROSPECTUS

There is a contrast between behavioral work on terrestrial and aquatic organisms: much terrestrial work is done in the field while aquatic animals are more likely to be watched in the laboratory. The reasons for the difference in study locale are obvious, the consequences are less so. Consider how little we would know of the diversity of hummingbirds, their behaviors, and their flowers if we constrained them to cages.

Behavioral/ecological studies of other fish senses indicate that we have much to learn about the use of the lateral line and the ecological and evolutionary contexts for behavior. I see two approaches. In one the researcher pursues fishes with unusual lateral lines and compares these to the closest possible relative without the specialization (a cladistic approach). An interesting recent example of an unusual lateral line is the connection between the lateral line and swim bladder in *Chaetodon* (Webb 1998); the fish and a close relative without the connection would be very worthy of behavioral study. In the second approach one chooses animals based on their ecology. Insights into how diverse the senses of vision, hearing, and the chemosenses are come from examination of animals from environments that place great evolutionary pressures on the organisms. For example, sculpins were developed as "models" because they tend to be nocturnal (Hoekstra and Janssen 1985). Probably the most interesting behaviors will come from fishes from very old and dark environments, not from the fishes readily accessible. The two approaches ultimately converge; the fishes of unusual environments are likely to have unusual sensory anatomies.

In the oldest of all ecosystems, the deep sea, we should expect to find examples of crypsis, mimicry, miss-direction of predators, etc. Unfortunately study is difficult where the lateral line is likely to be an important sense. None-the-less, important techniques are being developed. Bioacoustics using multiple sources can track individual pelagic predators and prey and infrared

Bathyclupea

Compethrus
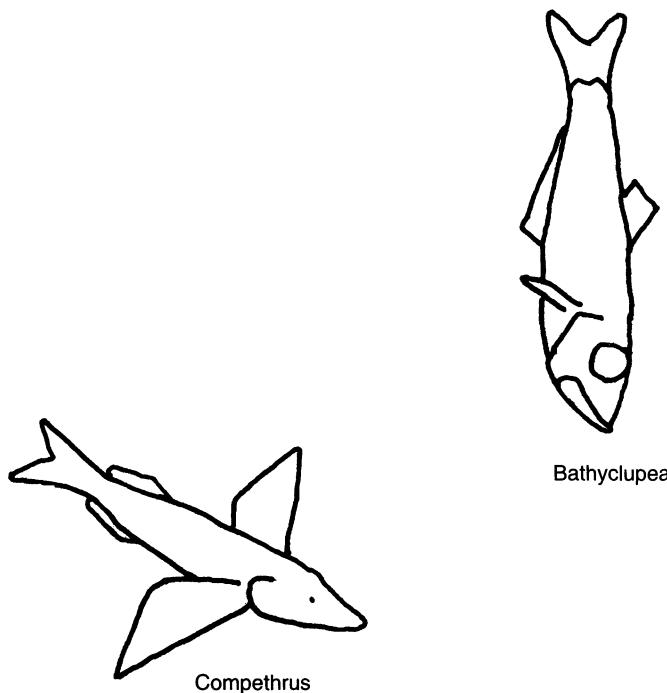
Fig. 10 Gliding/sinking fishes with membranous head lateral line canals. On the left is *Comephorus dybowskii* which swims up via its caudal fin with pectoral fins pressed along its body, then glides down diagonally with the pectoral fins acting as wings. On the right is *Bathyclupea* sp. which sinks head down, adjusting attitude with its pectoral fins. When its snout touches the bottom, it swims upward, tail-first (head remaining down), using its pectoral fins. Drawn from videotapes from Russian Academy of Sciences and Woods Hole Oceanographic Institution.

cameras allow some in situ observations (Collins and Hinch 1993). The general pattern is that evolution is usually more creative than our imagination. With each breakthrough in our ability to observe fish in the wild and in the dark there will be surprises. The discoveries being made on how zooplankton use and manipulate hydromechanics sets a precedent for intriguing examples among fishes (Yen and Strickler 1996). Much has been done to lay down the fundamentals of hydromechanical detection in fishes, this now needs to be better applied to ecologically meaningful situations.

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Neurobiology of the Fish Lateral Line: Adaptations for the Detection of Hydrodynamic Stimuli in Running Water

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ABSTRACT

All fishes possess a lateral line system, which serves as a receptor for hydrodynamic stimuli such as those generated by conspecifics, predators or prey. The lateral line is comprised of numerous individual sensory units, the neuromasts, which can occur freestanding on the surface of the skin or embedded in subepidermal canals. The morphological design of the peripheral lateral line can be quite different in different fish species and is thought to represent an adaptation to the hydrodynamic conditions that prevail in the habitat of a given species. However, despite gross morphological differences, the general physiology of the peripheral lateral line appears to be quite similar in different fish species at least when the system is studied under still water conditions. In contrast to the peripheral lateral line that has been studied extensively, much less is known about the processing of hydrodynamic information by lateral line neurons in the fish brain. In addition, not much is known about the processing of naturally occurring hydrodynamic stimuli. In order to understand how the lateral line functions under more natural stimulus conditions, complex water motions generated by moving sources have been used as lateral line stimuli and the system has been studied under running water conditions. This chapter summarizes the main result from these studies, which have, for the first time, revealed a clear form-function relationship for the lateral line system.

Key words: Lateral line, Mechanosense, Neuromast, Hydrodynamic, Background noise, Running water

INTRODUCTION

Water motions provide a wealth of sensory information that can be used by aquatic animals for orientation and communication. Water motions are generated by animate sources like conspecifics, predators or prey and by inanimate sources, for instance water currents, wind, and changes in temperature or gravity. To detect and analyze water motions, aquatic animals have evolved highly sophisticated hydrodynamic receptor systems (review: Bleckmann 1994). The hydrodynamic receptor system of fishes is the mechanosensory lateral line.

Numerous studies have addressed aspects of development, morphology, physiology and behavioral function of the lateral line (for reviews see e.g., Coombs et al. 1989; Coombs and Montgomery 1999; Bleckmann 1993, 1994, 1998; Montgomery et al. 1995; Janssen 2003, this volume). However, many important questions about lateral line function and its effects on fish behavior have not yet been completely answered. For instance, we still do not know much about biologically relevant lateral line stimuli, what aspects of natural stimuli the system receives, analyzes and encodes, whether stimulus features are mapped in the brain and what the mechanisms are that underlie the analysis of natural hydrodynamic stimuli.

Physiological and behavioral aspects of the lateral line have been studied extensively using a stationary sinusoidally vibrating sphere as a stimulus source (e. g. Coombs et al. 1989; Coombs et al. 1996; Münz 1985; Mogdans and Bleckmann 1999). The hydrodynamic stimulus generated by a vibrating sphere is well defined (Harris and van Bergeijk 1962), however, pure sine waves are rare in nature. Naturally occurring oscillatory water motions contain multiple frequencies and are modulated in frequency and/or amplitude (review: Bleckmann 1994). In addition, lateral line experiments typically have been performed under low noise conditions, i.e., the water in the experimental tank was maintained as still as possible. In nature, however, either the fish or the water surrounding the fish or both, fish and water are moving. Consequently, the lateral line is almost permanently exposed to running water conditions that may impair the detection of other hydrodynamic stimuli. Thus, in order to understand how the lateral line is adapted to the behavioral needs of an animal, one needs to study the system with more natural hydrodynamic stimuli applied in noisy environments.

A comprehensive description of peripheral lateral line designs and their relevance for various fish behaviors has been given in the previous chapter. Therefore, this chapter reviews only briefly morphology and biomechanical properties of lateral line receptors. The main part of the chapter summarizes recent data gathered in goldfish, *Carassius auratus*, and trout, *Oncorhynchus mykiss*, which describe how lateral line nerve fibers and lateral line neurons in the brainstem respond to hydrodynamic stimuli that were presented to the fish in a noisy environment, i.e., in running water. Detailed information about the anatomy and physiology of higher lateral line brain centers can be obtained from other reviews (e.g., Bleckmann 1994; Coombs and Montgomery 1999; Mogdans and Bleckmann 2001).

MORPHOLOGY OF THE LATERAL LINE PERIPHERY

The lateral line is comprised of numerous individual sensory units, the neuromasts. Two types of neuromasts can be distinguished (Fig. 1): superficial neuromasts which occur freestanding on the skin, in pits, or on pedestals raised above the skin, and canal neuromasts which are located in subepidermal canals that are in contact with the water through canal pores. Typically a canal neuromast is located halfway between two adjacent pores.

Both types of neuromast have the same basic bauplan. They consist of sensory hair cells, supporting cells and mantle cells under a gelatinous cupula (Fig. 1B-D). The cupula connects the hair cell's ciliary bundles with the surrounding water (Münz 1979). Neuromast shape is circular or elliptical with maximal diameters ranging between less than 100 μm up to more than
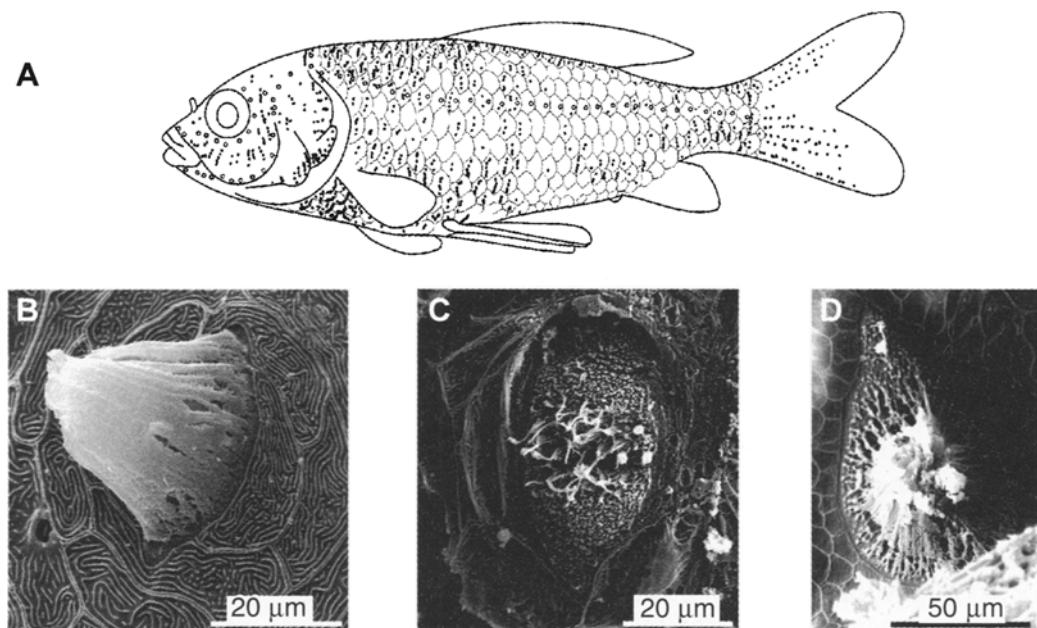
Fig. 1 Morphology of the lateral line periphery. **A** Distribution of neuromasts in the goldfish, *Carassius auratus*. Dots indicate superficial neuromasts, circles represent canal pores. Drawing gratefully provided by A. Grotefeld. **B-D** Electron micrographs of goldfish neuromasts. **B** Superficial neuromast with intact cupula. **B** Superficial neuromast with removed cupula exposing the ciliary bundles of the sensory hair cells. **C** Canal neuromast with intact cupula. Note the differences in scale.

600 μm. Consequently, the number of hair cells within a neuromast ranges from very few (e.g. Münz 1979; Song and Northcutt 1991) up to several thousand (Müller 1984). Superficial neuromasts are generally smaller and have fewer hair cells than canal neuromasts (Münz 1989). Each neuromast contains two populations of hair cells with oppositely oriented ciliary bundles (Flock and Wersäll 1962). Afferent nerve fibers innervate only hair cells of identical orientation (Görner 1963). Consequently, lateral line neuromasts are bidirectionally sensitive due to the separate innervation of unidirectionally polarized hair cells (Flock 1965; Kroese and Netten 1989).

BIOMECHANICAL PROPERTIES OF THE LATERAL LINE

The adequate stimulus for a lateral line neuromast is fluid movement along the cupula. This fluid movement cause the cupula to slide across the underlying sensory epithelium (Netten and Kroese 1987), which results in shearing motions of the ciliary bundles. The stiffness of the coupling of the cupula to the sensory epithelium depends on cupula size and on the pivoting stiffness of the ciliary bundles of the hair cells and this in turn depends on the number of hair cells within a neuromast (Denton and Gray 1989; Netten et al. 1990). The fluid forces that move the cupula consist of both viscous and inertial components. The relative contribution of

these two components varies with frequency and is related to the frequency-dependent boundary layer around the cupula (Netten and Kroese 1987). Within the frequency range relevant for the lateral line, small diameter cupulae will be more affected by viscous forces whereas large diameter cupulae will be more affected by inertial forces (Netten and Kroese 1989).

Superficial neuromasts are generally smaller than canal neuromasts. They are driven primarily by viscous drag forces that are proportional to the velocity of the water flowing along their sides (Kalmijn 1989). Thus, superficial neuromasts function as velocity detectors. In contrast, canal neuromasts are pressure gradient detectors because fluid flow within lateral line canals occurs only as a consequence of a pressure gradient between canal pores. Outside the canal, the pressure gradient is proportional to the acceleration of the water. Thus, canal neuromasts may be considered acceleration detectors with respect to the water motions outside the canal (Kalmijn 1989). For a given displacement, velocity and acceleration increase with increasing frequency. Consequently, canal neuromasts are driven more effectively with increasing frequency of an external water displacement. In other words, lateral line canals represent high-pass filters for hydrodynamic stimuli (Denton and Gray 1988; Bleckmann and Münz 1988).

VARIATIONS IN LATERAL LINE DESIGN

The morphological design of the peripheral lateral line can be quite different in different fish species (e.g. Coombs et al. 1988; Webb 1989; Janssen 2003, this volume). These differences are believed to represent at least in part adaptations to the hydrodynamic conditions prevailing in the habitat of a species. There are several possibilities by which the lateral line can be adjusted to the sensory conditions under which an animal lives. For instance, with increasing width of a lateral line canal, its high-pass characteristic is shifted towards lower frequencies (Denton and Gray 1989). In some species, a membrane-like covering on a lateral line canal adds resonance thereby enhancing sensitivity to a particular frequency band (Denton and Gray 1989). The sensitivity and frequency characteristic of the lateral line can be modified by altering the number and size of canal pores (Bleckmann and Münz 1990), cupula radius, cupula length (due to boundary layer effects), cupula sliding stiffness (which depends mainly on the number of hair cells within a neuromast), and the density of the fluid surrounding the cupula (e.g. in a lateral line canal). Other ways to modify the hydrodynamic information reaching the brain include the variation of the number and placement of superficial neuromasts and/or the number and design of lateral line canals (see also Janssen 2003, this volume).

NATURAL HYDRODYNAMIC STIMULI

Natural hydrodynamic stimuli that may be relevant for lateral line perception can occur at the water surface or in midwater. Surface waves are caused by terrestrial insects fallen into the water or by aquatic animals contacting the water-air interface in order to breathe or feed (Bleckmann 1988). They can be detected by the specialized head lateral line systems of surface-feeding fishes.

Midwater stimuli are caused by fishes or other aquatic animals. Swimming zooplankton and fish body vibrations generate oscillatory water movements that contain frequencies from DC up

to about 45 Hz (Enger et al. 1989; Kirk 1985; Montgomery 1989; Satou et al. 1991). Subundulatory swimming fish generate a trail of vortices (Blickhan et al. 1992) that contains frequencies up to at least 100 Hz (Bleckmann et al. 1991). The hydrodynamic trail produced by a swimming fish may last for up to several minutes (Hanke et al. 2000) thus making it possible that predatory fish track the wakes generated by prey fishes (Pohlmann et al. 2001).

Hydrodynamic stimuli may be self-generated, for instance by the fish's own swimming or breathing movements. This may be advantageous or disadvantageous for the individual. The blind cave fish, Anoptichthys jordani, provides a well-studied example how self-generated stimuli can be used to obtain information about the environment. While swimming, the fish generates a flow field around its body that is altered if the fish passes an object and these alterations can be sensed by the lateral line. In this way, swimming or gliding fish can obtain lateral line information about nearby stationary objects (e.g., Campenhausen et al. 1981; Hassan 1989).

Hydrodynamic stimuli generated by breathing movements (Fig. 2) are disadvantageous for the individual since the may be detected by predators and/or interfere with the detection of novel relevant stimuli and in this respect can be considered as hydrodynamic noise. Strategies to avoid this hydrodynamic noise can be seen in fish behavior (i.e., fish cease to breath if they are confronted with new and possibly dangerous stimuli) and lateral line morphology (i.e., placement of the trunk lateral line canal relative to the operculum or the fins). Some fish posses the ability to cancel self-generated noise by means of a reafferent signal that is subtracted in the fish brainstem from the information that is conveyed to the brain by primary afferent fibers (Montgomery et al. 1997).

Abiotic sources for hydrodynamic stimuli are water currents, wind, changes in temperature, salinity gradients and gravity (e.g., Wetzel 1983). A fish that lives in a pond, lake, or the deep ocean probably faces little hydrodynamic noise. In contrast, a fish that lives in a rapidly running river or along the ocean-shoreline faces turbulent water conditions that interfere with lateral line perception. Although these stimuli are generally considered as noise, they nonetheless may convey important information. For instance, running water may be used by the lateral line to mediate rheotaxis (Montgomery et al. 1999) but at the same time, as described in detail below, interferes with the ability of the lateral line system to detect hydrodynamic stimuli (Engelmann et al. 2000, 2002).

PHYSIOLOGY OF THE LATERAL LINE PERIPHERY

Responses to Sine Wave Stimuli Under Still Water Conditions

The physiology of the peripheral lateral line has been studied extensively in still water using single-frequency sinusoidal wave stimuli. As a monitor for neuromast function the activity of primary afferent nerve fibers has been recorded (e.g., Münz 1985; Coombs et al. 1996). Despite variations in morphology, the physiology of the peripheral lateral line turned out to be quite similar in different fish species. Primary afferent fibers are spontaneously active (e.g. Hoagland 1933; Sand 1937; Topp 1983; Montgomery et al. 1996). A constant-amplitude sine wave stimu-
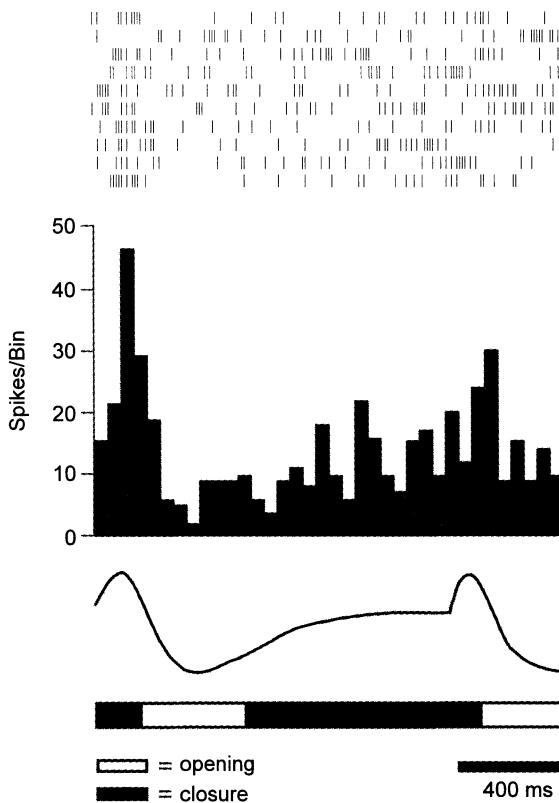
Fig. 2 Activity of a goldfish posterior lateral line nerve (PLLN) fiber in relation to the fish's breathing activity. Top: Raster diagram of the neural activity recorded across ten breathing cycles (each marker represents one action potential). Middle: Corresponding peri-stimulus-time histogram (binwidth 50 ms). Bottom: Voltage output of a dielectric element representing the movements of the ipsilateral operculum. Horizontal bar indicates periods of opening (open bars) and closure (filled bars) of the operculum.

lus causes a sustained increase in firing rate (e. g. Mogdans and Bleckmann 1999; Fig. 3A). Fibers respond to frequencies of less than 1 Hz up to about 150 Hz. Within this frequency range, a peak-to-peak displacement of 0.01 µm of the water at the surface of the skin can be sufficient to cause a neural response. At low stimulus amplitudes discharges are weakly phase-coupled to the sinusoidal wave stimulus. With increasing stimulus amplitude phase-coupling increases until it reaches saturation (Fig. 3B). Thus, the amplitude of a sine wave stimulus is encoded by the degree of phase-coupling. In addition, stimulus amplitude is encoded by firing rate, which also increases with increasing stimulus amplitude. However, firing rates increase above spontaneous activity only at stimulus levels that are about three times higher than those causing strong phase-locking (Schellart and Kroese 1992; Mogdans and Bleckmann 1999; Fig. 3B).

Using a vibrating sphere stimulus, two types of afferent fibers can be distinguished. One type of afferents responds with a doubling of discharge rate to a doubling of stimulus frequency. This is equivalent to a gain of 6 dB/octave. In addition, action potentials show a phase lead with
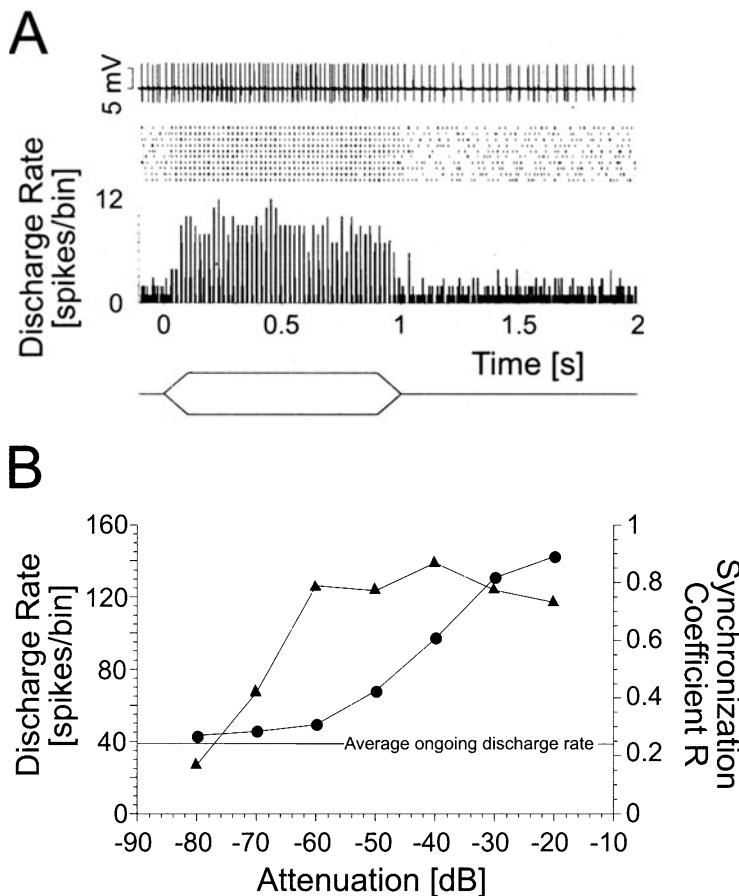
Fig. 3 Physiology of the lateral line periphery. **A** Raster diagram and peri-stimulus-time histogram (binwidth 2 ms) of the responses of a goldfish posterior lateral line nerve (PLLN) fiber to ten repetitions of a 50 Hz sinc wave stimulus generated by a stationary vibrating sphere (sphere displacement 4 μm peak-to-peak, sphere diameter 8 mm). Top trace: original recording; bottom trace: stimulus. **B** Input-output function of a PLLN fiber. >Discharge rates (circles, left-hand axis) and synchronization coefficient R (triangles, right-hand axis) are plotted as a function of stimulus level (in rel. dB, (−20dB = 42.5 μm sphere displacement).

respect to sphere displacement of about 90 degrees for frequencies ≤ 10 Hz (e.g. Münz 1985; Kroese and Schellart 1989, 1992; Montgomery and Coombs 1992; Montgomery et al. 1994). A second type of afferents responds with a fourfold increase in discharge rate to a doubling of stimulus frequency, i.e., a gain of 12 dB/octave. In this type, action potentials show a phase lead of about 180 degrees (Kroese and Schellart 1992). Gains of 6 dB/octave and 12 dB/octave are a consequence of the fact that velocity and acceleration are the first and second derivative (re time) of the displacement. Thus, based on theoretical (Kalmijn 1989) and biomechanical (Netten and Kroese 1987) considerations (see above), afferents of the first type—which respond about proportional to water velocity-innervate superficial neuromasts whereas afferents of the second type—which respond about proportional to water acceleration-innervate canal neuromasts.

Responses to Complex Wave Stimuli Under Still Water Conditions

Sine wave stimuli have been used to characterize general physiological properties of the peripheral lateral line, like threshold, phase-coupling ability, and dynamic amplitude range. However, sine waves are highly unnatural. A complex spatial and temporal pattern of water flow characterizes natural hydrodynamic stimuli like those generated by swimming fish (e.g., Blickhan et al. 1992; Bleckmann et al. 1991). Swimming goldfish, for example, generate a complex hydrodynamic trail that may be detected with particle imaging velocimetry (PIV) for up to 30 s after the fish passed by (Hanke et al. 2000). To better understand how natural stimuli are represented by the lateral line periphery, primary afferent activity has been studied using wave stimuli generated by small objects that were passing the fish (e.g., Bleckmann and Zelick 1993; Mogdans and Bleckmann 1998). The water motions generated by a moving object are changing both in time and space resulting in a complex pattern of water flow across the fish surface (Hanke and Bleckmann 1999; Mogdans et al. 1999).

Afferent fibers in the posterior lateral line nerve of goldfish respond to a moving object with a pattern of discharge that consists of excitation followed by inhibition or vice versa (Mogdans and Bleckmann 1998; Figs. 4 and 7A). The sequence of excitation and inhibition inverses when object motion direction is reversed. This can be predicted from the intrinsic directional sensitivity of the hair cells within a neuromast. About 70% of the nerve fibers discharged numerous unpredictable bursts of spikes after an object had passed the fish (Fig. 4A). These bursts are probably caused by the wake of the moving object, which is characterized by fairly large and unpredictable changes in water velocity. About 27% of the fibers did not continue to respond after the object had passed the fish (Fig. 4B). Afferent fibers of the first type most likely receive input from superficial neuromasts which are highly sensitive to water velocity (e.g. Kroese and Schellart 1992). Canal neuromasts, in contrast, are more sensitive to water acceleration which is proportional to pressure gradient. Pressure gradients are prominent mainly when the object passes the fish and are small in the object's wake (Mogdans and Bleckmann 1998). Thus, fibers of the second type most likely receive input from canal neuromasts.

Sensitivity to Running Water

Over the last 40 years researchers tried to understand the lateral line by studying its function under still water conditions. However, as pointed out in the introduction, still water conditions are very unnatural since fishes are mostly subject to some kind of background noise. For example, fishes that live in rivers are almost permanently exposed to running water. Other fishes, for instance species that live in the ocean surf, are subject to highly turbulent water motions. Even in habitats in which the water is barely moving, like tide pools or freshwater ponds, fishes that are swimming around are permanently exposed to water flow as a consequence of their own swimming movements.

The responses of primary afferent fibers to running water have been recorded in flow tanks in which a fish was exposed to a constant laminar water flow (Engelmann et al. 2000, 2002; Voigt
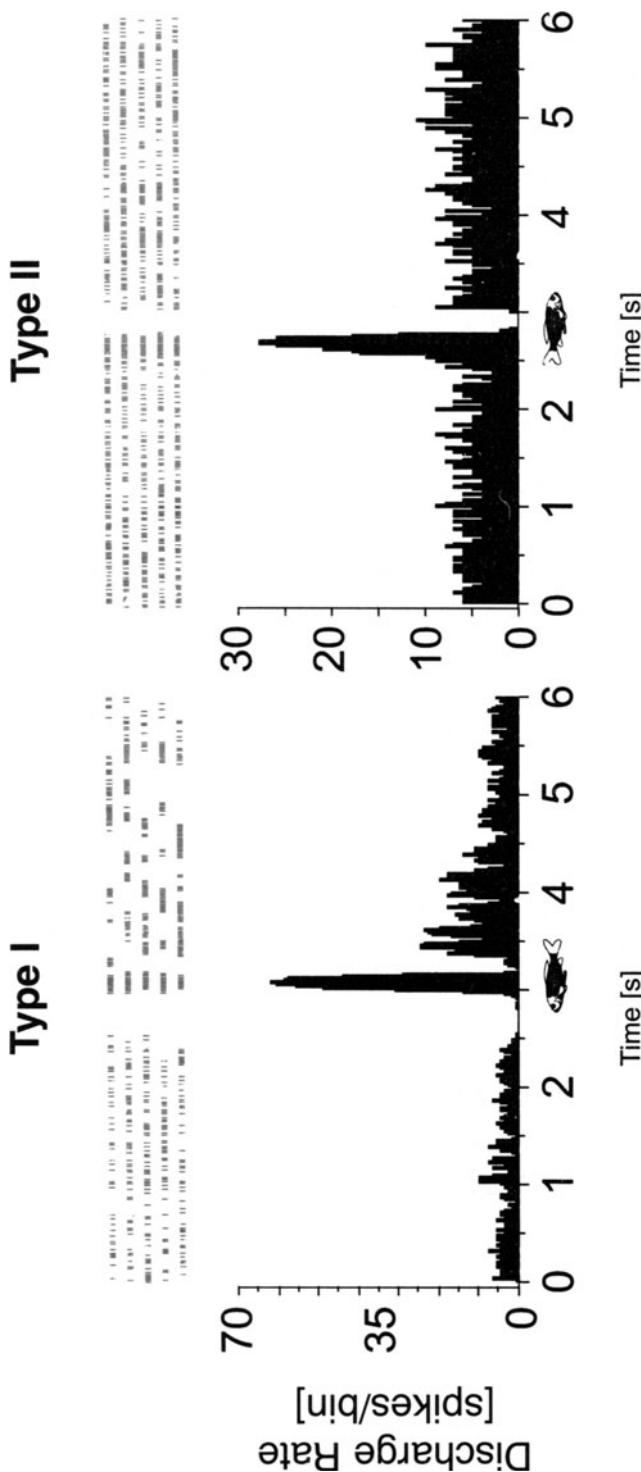
Fig. 4 Peripheral lateral line responses to a moving object (Plexiglas rod, square cross section of 1x1 cm, speed 15 cm/s). Raster diagrams of the responses to five object presentations and corresponding peri-stimulus-time histograms (binwidth 20 ms) of two PLLN fibers in the goldfish are shown. Fish symbols represent location, orientation and size of the fish relative to the path of the moving object. Left Response of a type I unit to the object moving from anterior to posterior. The unit responded with inhibition followed by excitation and again inhibition at about the time when the object was closest to the fish. It continued to fire unpredictable bursts of spikes after the object had passed along the side of the fish. Right Response of a type II unit to the object moving from posterior to anterior. The unit responded with excitation followed by inhibition but barely responded after the object had passed along the side of the fish.

et al. 2000; Carton 2002). In these experiments, Engelmann et al. (2000, 2002) again distinguished two types of primary afferent fibers: type I fibers and type II fibers (Fig. 5). Type I fibers responded to the water flow with an increase in discharge rate for as long as it was maintained. In contrast, type II fibers did not change their discharge rate in response to the water flow. The continuous firing of type I fibers suggests that they were innervating superficial neuromasts, which are continuously stimulated by the background flow. Type II fibers most likely were innervating canal neuromasts, which are unresponsive to background flow since a laminar flow does not create pressure differences between canal pores.

Responses to Sine Wave Stimuli Under Running Water Conditions

Engelmann et al. (2000, 2002) studied the responses of primary afferent fibers to sine wave stimuli presented in running water. These experiments were done in goldfish, *Carassius auratus*, and rainbow trout, *Oncorhynchus mykiss*, two species that differ in habitat and lateral line morphology. Goldfish are relatively slow swimming fish that live in still waters whereas trout are fast swimming fish that are found predominantly in running water. In goldfish the arrangement (size and orientation) of canal neuromasts is typical for fishes with widened lateral line canals (Engelmann et al. 2002; Webb 1989), whereas canal neuromast arrangement in trout is more typical for fishes with narrow canals (Webb 1989). Another difference is that goldfish have
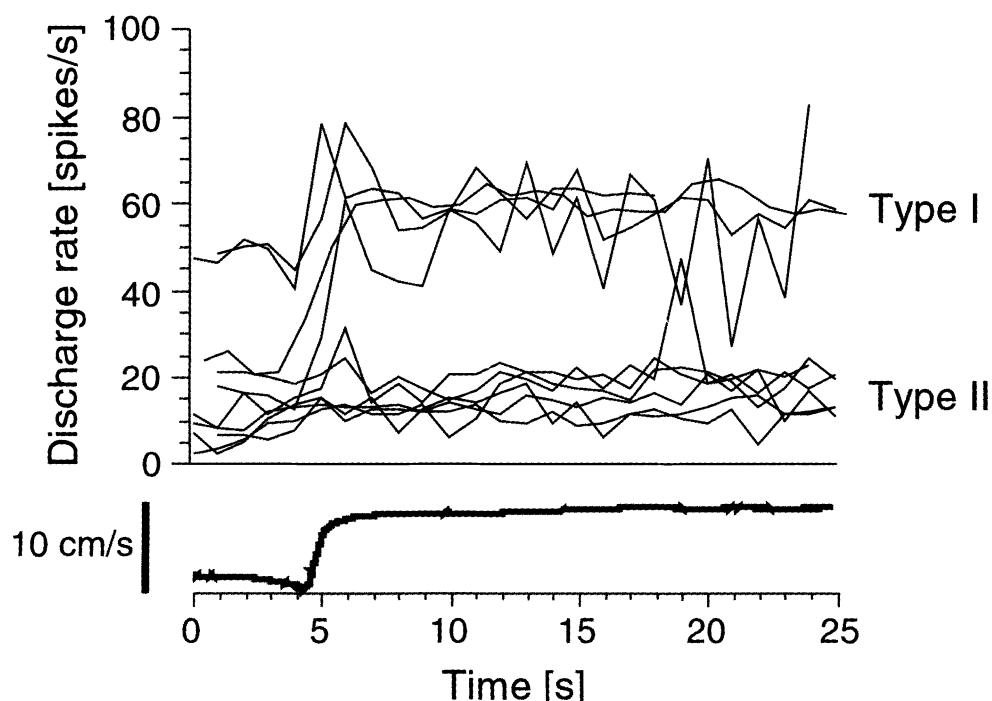
Fig. 5 Responses of goldfish type I and type II posterior lateral line nerve fibers to running water (velocity 10 cm/s). Bottom trace represents the free field water velocity in the experimental tank.

several hundred superficial neuromasts on each side of the body (Puzdrowski 1989; Engelmann et al. 2002) whereas trout have only few superficial neuromasts (Disler 1979; Engelmann et al. 2002).

In both goldfish and trout the responses of type I and type II primary afferents to sine wave stimuli were affected in a different way by background running water (Fig. 6). In still water, type I and type II afferents exhibited sustained and phase-locked responses that could hardly be distinguished from each other. However, in a flow of 10 cm/s a clear difference emerged. Whereas the responses of type I fibers to a sinusoidal water motion were masked, the responses of type II fibers were hardly affected by the flow. This demonstrates a clear functional difference between the superficial and the trunk canal neuromast system. Superficial neuromasts are continuously stimulated by running water as indicated by their increased firing rates. As a consequence, type I afferents respond with high sensitivity to a vibrating sphere only in still water, i.e., if the fish is not exposed to a background water flow. Trunk canal neuromasts, in contrast, are hardly affected by running water and thus type II afferents respond about equally well to a vibrating sphere in still and running water. Obviously, superficial and canal neuromasts are affected in a similar manner by running water in two species that have different life styles, are subject to different hydrodynamic conditions in their respective habitat, and have different lateral line designs.
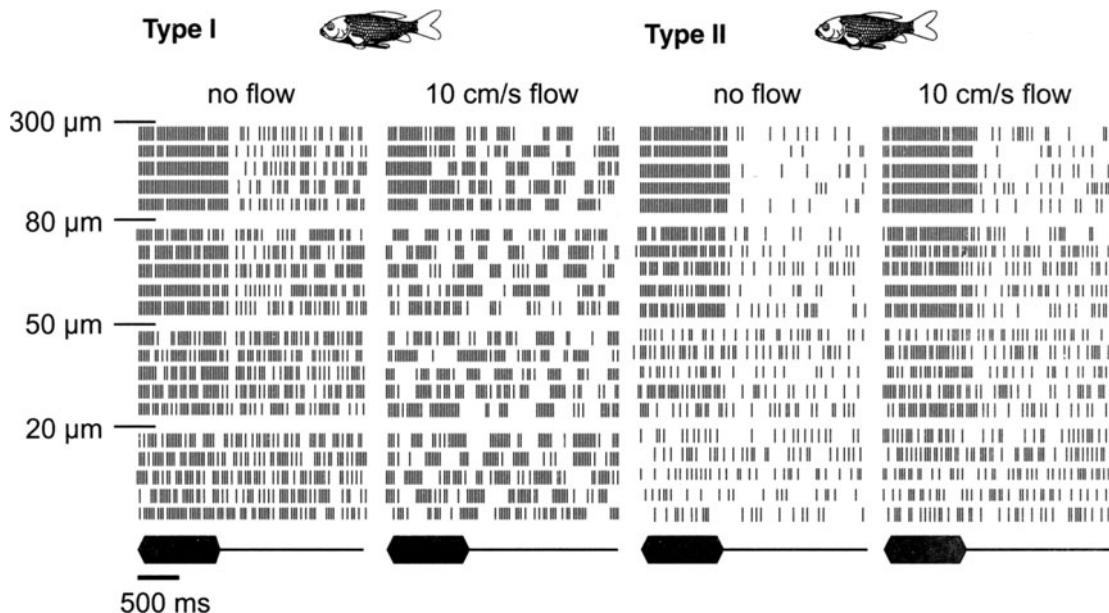
Fig. 6 Responses of goldfish type I and type II posterior lateral line nerve fibers to a 50 Hz sine wave stimulus generated by a vibrating sphere of 10 mm diameter. Raster diagrams of the responses to five stimulus repetitions are shown for four peak-to-peak displacements. Data were recorded in still water (no flow) and in running water (velocity 10 cm/s). Flow direction was from anterior to posterior. The background flow masks the responses of the type I fiber but not that of the type II fiber.

Responses to Complex Wave Stimuli Under Running Water Conditions

Although sine wave stimuli can reveal differences in function between superficial and canal neuromasts in running water, one needs to ask how the system's responses to more complex and thus more natural water motions are affected by running water. It turned out that the effects of running water on afferent fiber responses to a moving object differed from those on the responses to a sine wave stimulus.

In goldfish, running water clearly masked the responses of type I fibers to the moving object but the strength of the effect depended on object motion direction (Fig. 7). When object motion direction was from anterior to posterior alongside the fish, i.e., in the same direction as the flow, responses of type I fibers were masked (Fig. 7, left columns). When object motion direction was from posterior to anterior alongside the fish, i.e., opposite to the direction of the flow, the responses of only some type I fibers were masked. Most type I fibers exhibited a discernible response to posterior-anterior object motion even in running water. However, the temporal pattern that was characteristic for the still water condition was far less distinct (Fig. 7, right columns). In contrast to the responses of type I fibers, responses of type II fibers to the moving object were less affected by running water. Nevertheless, in the few type II goldfish fibers that were tested so far, responses to anterior-posterior object motion in running water were weaker compared to still water whereas responses to posterior-anterior object motion were more or less the same in still and in running water.
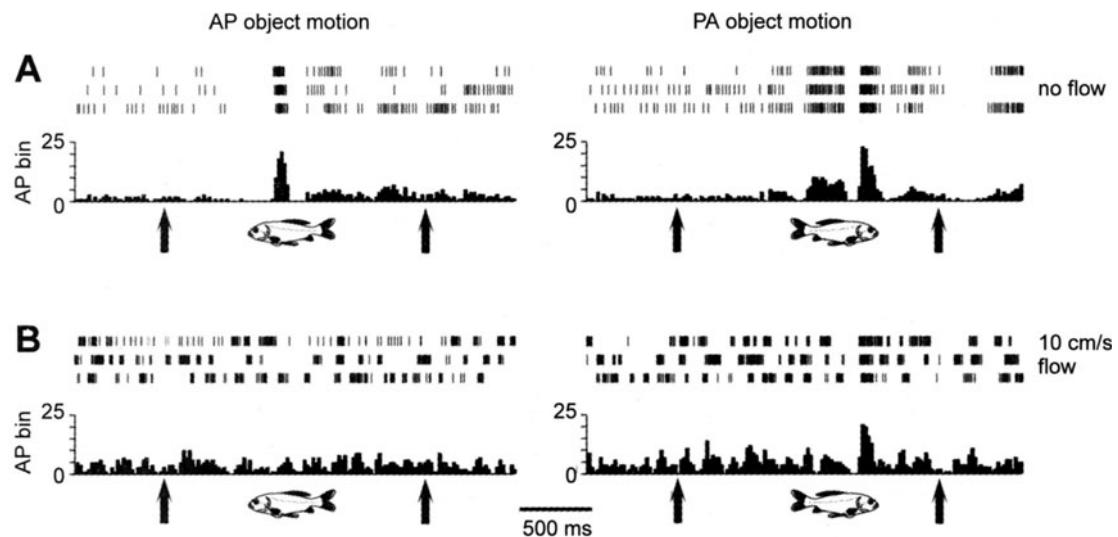

Fig. 7   Peripheral lateral line responses to a moving object (sphere, 8 mm diameter, speed 15 cm/s) in still and running water. Raster diagrams of the responses to three object presentations and the corresponding peri-stimulus-time histograms (binwidth 20 ms) of a goldfish PLLN fiber are shown. Motion direction was from anterior to posterior (AP, left) or from posterior to anterior (PA, right). Fish symbols represent location, orientation and size of the fish relative to the path of the moving object. **A** Response to the moving object in still water. Compare responses with those shown in Fig. 4. **B** Response to the moving object in running water (10 cm/s flow velocity). Note the increased discharge rate indicating that this fiber most likely received input from a superficial neuromast.

In trout, the effects of running water on the responses of afferent fibers to a moving object were weaker than those observed in goldfish. However, in trout, all but two units tested so far were type II units, i.e., they were insensitive to running water. The responses of most of these units to anterior-posterior object motion were comparable in still and running water or only slightly weaker in running water.

The effects of running water on the responses to a moving object can be explained to a large degree by peripheral hydrodynamic effects. Measurements of subsurface pressure using a hydrophone and of water velocity using a hot-wire anemometer showed that in still water the moving object generated changes in pressure and water velocity that were to a large degree independent of object motion direction. In running water, however, the hydrodynamic signals generated by the moving object interfered with those generated by the running water in a direction-dependent manner. When the object was moved with the flow, changes in pressure and water velocity generated by the moving object were masked. When the object was moved against the flow, only a transient change in water velocity was discernable from the increased background water velocity whereas the change in pressure (and thus any associated change in pressure gradient) was even greater than the pressure change measured in still water. These findings are by large in agreement with the physiological data. Responses to the moving object of velocity-sensitive type I afferents, which were more abundant in goldfish than in trout, were masked by running water in a direction-dependent manner, i.e., masking was stronger when the object moved with the flow than when it moved against the flow. In contrast, responses of pressure gradient-sensitive type II afferents, which were more abundant in trout than in goldfish, were nearly unaffected by running water when the object moved with the flow but were even greater when the object moved against the flow.

PHYSIOLOGY OF THE LATERAL LINE BRAINSTEM

Responses to Hydrodynamic Stimuli Under Still Water Conditions

To understand how the information that is received by the peripheral lateral line is analyzed by the fish's brain the activity of central lateral line neurons must be studied. The first site of sensory integration in the ascending lateral line pathway is the brainstem medial octavolateralis nucleus (MON) (e.g. McCormick and Hernandez 1996; New et al. 1996). Sensory information reaches the MON via at least three distinct lateral line nerves (e.g., Northcutt 1989; Puzdrowski 1989; Song and Northcutt 1991). Output neurons of the MON project predominantly to the contralateral midbrain torus semicircularis (e.g., McCormick & Hernandez 1996). From there the ascending lateral line pathway extends to the diencephalic lateral preglomerulosus nucleus and finally reaches the area dorsalis of the telencephalon (e.g., Murakami et al. 1986).

Compared to afferent fibers, MON units have lower spontaneous and evoked rates of activity. The responses to sine wave stimuli exhibit greater degrees of adaptation and greater heterogeneity both in terms of the response patterns and in terms of phase-coupling. Moreover, MON units are substantially less sensitive to sine wave stimuli than primary afferents (e.g., Paul and Roberts 1977; Caird 1978; Wubbels et al. 1993; Montgomery et al. 1996; Coombs et al. 1998). In

goldfish, about 30% of the units in the MON did not respond to a stationary vibrating sphere, even when tested with displacement amplitudes of up to 800 μm (Mogdans and Goenechea 2000). Such displacement amplitudes are substantially higher than those causing rate saturation in lateral line afferents. However, many of these seemingly insensitive units readily respond to the water motions generated by a moving sphere. Receptive fields of MON units can be fairly large and complex consisting of areas from which a sine wave stimulus causes excitation and adjacent areas from which a sine wave stimulus causes suppression of the ongoing discharge rate (Mogdans and Kröther 2001).

The responses of MON units to moving object stimuli are very diverse (Mogdans et al. 1997). Nevertheless, two response types can be distinguished. Like type I afferents, many MON units respond to the passing object and to the water motions generated in the wake of it. These units probably receive input from superficial neuromasts. Other MON units, similar to type II afferents, respond with a transient increase in discharge rate only.

Sensitivity to Running Water

The sensitivity of MON units to a constant background water flow has been studied in a flow tank (Kröther et al. 2002). As with primary afferents, two types of MON units were distinguished, i.e., units that were flow-sensitive and units that were insensitive to flow (Fig. 8). In running water, ongoing discharge rates of flow-sensitive units differed from those in still water and, in most cases, either increased or decreased with increasing flow velocity. Some units exhibited transient increases or decreases in discharge rate to the onset of the flow. Discharge rates of flow-insensitive units, in contrast, were not different for still and running water conditions. A reasonable hypothesis that can be deducted from these data is that flow-sensitive MON units receive input from superficial neuromasts, whereas flow-insensitive MON units receive input from canal neuromasts. However, the following data argue against the idea of a completely separate processing of superficial and canal neuromast input by the fish brain.

Responses to Hydrodynamic Stimuli Under Running Water Conditions

When goldfish were stimulated with a sinusoidally vibrating sphere in the presence of background flow, the responses of flow-sensitive and flow-insensitive MON units were affected differently. Depending on whether or not unit responses to the vibrating sphere were masked in running water, four unit types were distinguished: type MI, MII, MIII and MIV (Fig. 9). Type MI units were flow-sensitive. The responses of type MI units to a sine wave stimulus were masked by running water, i.e., either response rates or the degree of phase-coupling were decreased compared to still water conditions. This supports the assumption that type MI units received input from type I afferents, i.e., from superficial neuromasts. Type MII units, in contrast, were flow-insensitive. The responses of type MII units to sine wave stimuli were not altered under running water conditions. This supports the idea that type MII units received input from type II afferents, i.e., from canal neuromasts.
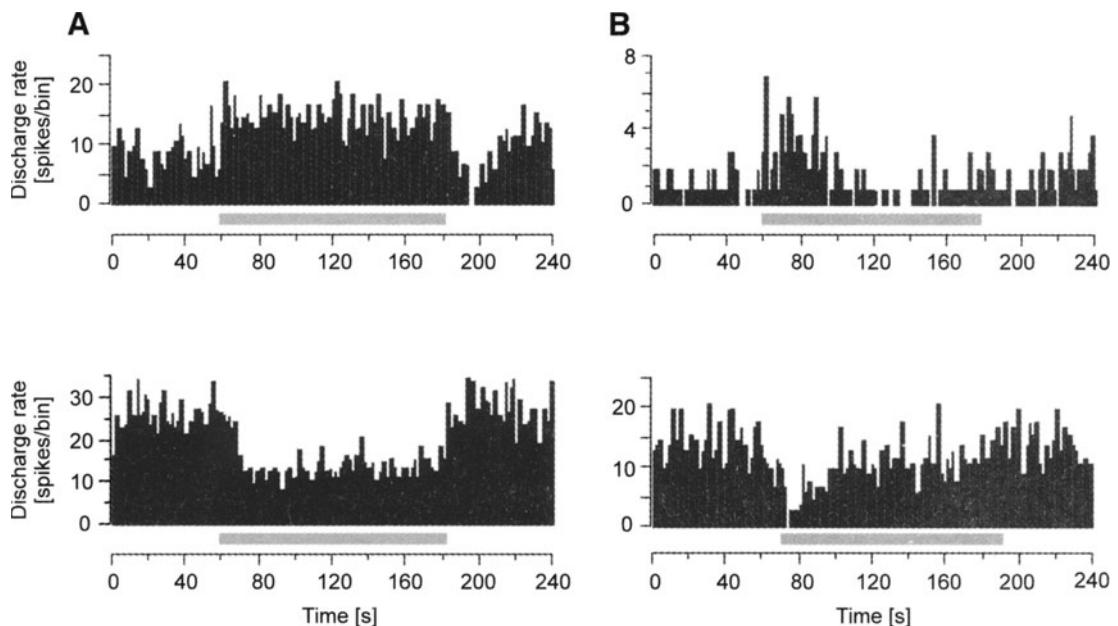
Fig. 8 Peri-stimulus time histograms (binwidth 1 s) of the activity of single MON units stimulated with a water flow of 15.5 cm/s. The stimulus trace below each diagram indicates flow duration. **A** Examples of two units that showed sustained increases (upper) and decreases (lower) in discharge rate as long as the water was flowing. **B** Example of two units that responded to the onset of water flow with a transient increase (upper) or decrease (lower) in discharge rate.

The response behavior of type MIII and type MIV units was different from that of type MI and type MII units. Like type MII units, type MIII units did not respond to running water. However, their responses to a vibrating sphere in background flow were significantly reduced. In contrast, type MIV units were flow-sensitive. However, their responses to a vibrating sphere were not affected by running water. These findings suggest that most likely, type MIII and MIV units receive input from both, superficial and canal neuromasts.

When a moving object was used as a stimulus source, the effects of running water on the responses of MON units were diverse and depended on object motion direction (Fig. 10). In goldfish, nearly all flow-sensitive units that responded to the moving object in still water did not respond or responded only weakly when the object was moved in running water along the side of the fish. Interestingly, a few flow-sensitive units responded to the moving object under running water conditions even though these units did not respond to the object in still water. Most of the flow-insensitive MON units studied so far did not respond to AP object motion in running water. However, all of these units responded to PA object motion in both still and in running water. Comparing neural responses with pressure measurements and measurements of water velocity suggests that many of the observed effects of running water on MON responses can be explained, like those of primary afferents, by peripheral hydrodynamic effects.
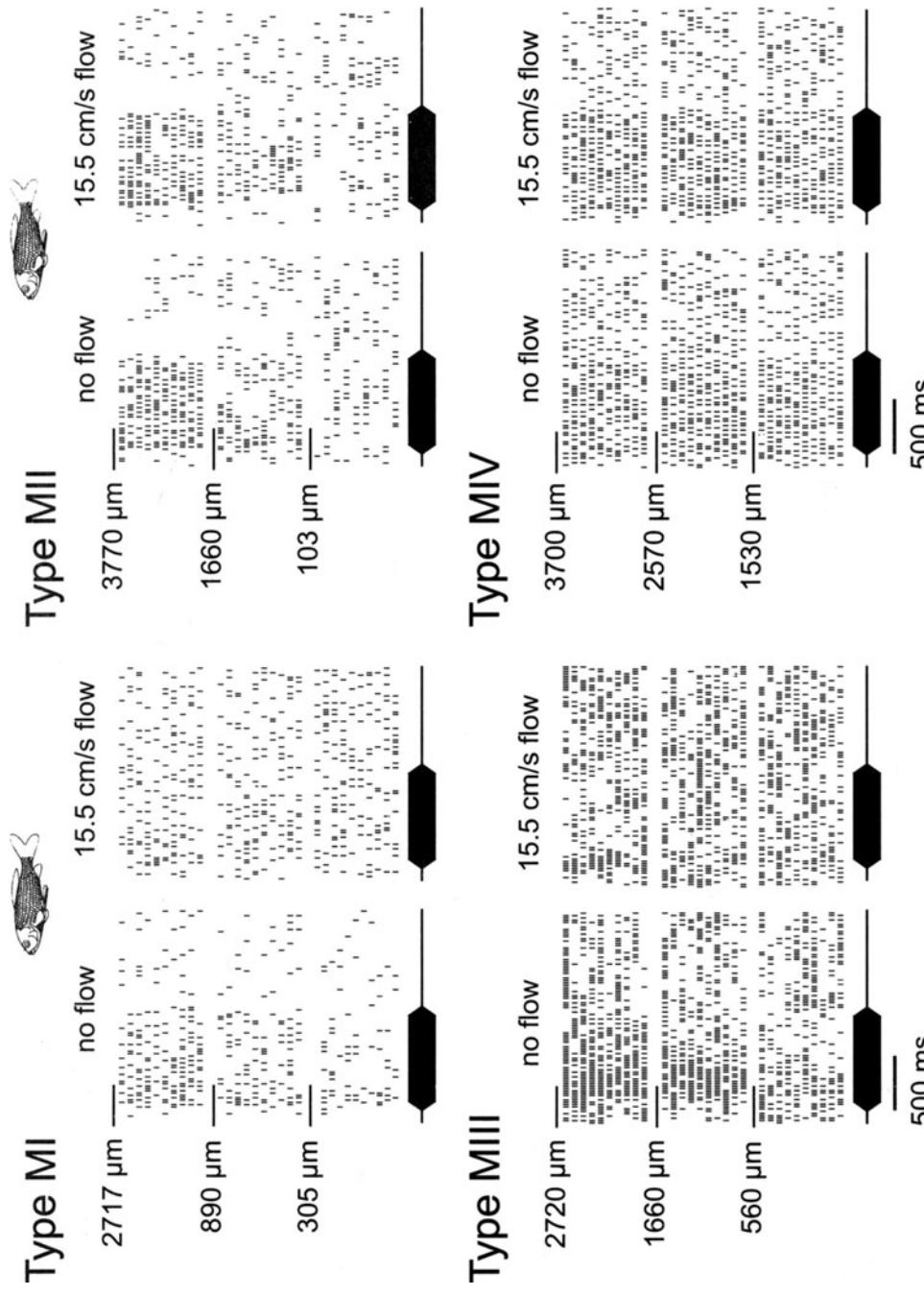
Fig. 9 Responses of goldfish type MI, MII, MIII and MIV cells in the medial octavolateralis nucleus to a 50 Hz sine wave stimulus generated by a vibrating sphere of 8 mm diameter. Raster diagrams of the responses to ten stimulus repetitions are shown for three peak-to-peak displacements. Data were recorded in still water (no flow) and in running water (velocity 15.5 cm/s). Flow direction was from anterior to posterior. The background flow masks the responses of the type MI and MIII cells but not that of the type MII and MIV cells.

CONCLUSIONS

The mechanosensory lateral line of fishes consists of two morphologically different subsystems that differ not only in morphology but due to different biomechanical properties, also in physiology. Whereas the superficial neuromast system is sensitive to water velocity, the canal neuromast system responds to pressure gradients and thus to water acceleration (Kroese and Schellart 1992; Coombs et al. 1996).

Under still water condition, both the superficial and the canal neuromast systems respond about equally well to the sinusoidal water motions generated by a stationary vibrating sphere. First evidence for a difference in performance of the two systems comes from experiments in which the lateral line was stimulated with more complex but also more natural water motions like those generated by a moving object. Superficial neuromasts respond with a series of unpredictable bursts of spikes to the water motions generated in the wake of the object. In contrast, canal neuromasts hardly show any bursting behavior since pressure gradients in the object's wake are negligible.

A clear difference between the two subsystems becomes apparent when the lateral line is studied under background noise conditions, i.e., under running water conditions. Whereas superficial neuromast function is substantially impaired in running water, canal neuromast function is not affected (Engelmann et al. 2000, 2002). Thus there is a clear form-function relationship for the lateral line that has been predicted first by Dijkgraaf (1963) but since then has not been demonstrated experimentally. For example, many fishes that live in running water or are fast swimmers tend to have extended lateral line canals and canal specialization (i.e. secondary and tertiary branching), but few superficial neuromasts (Bleckmann and Münz 1990), whereas species that live in still waters and are slow swimmers or have a sedentary behavior often have reduced, simple canals and a large number of superficial neuromasts (Bleckmann and Münz 1990; Dijkgraaf 1963; Puzdrowski 1989; Vischer 1990). In running water and/or when a fish is swimming, superficial neuromasts are permanently stimulated by water flow. As a consequence, superficial neuromasts become nearly useless for the detection of small and locally presented objects like small oscillating prey items. One possible function of the superficial neuromast system may be to mediate rheotaxis, i.e., the behavioral orientation to water currents (Baker and Montgomery 1999; Montgomery et al. 1997). In contrast, the canal neuromast system is perfectly suited for the detection and localization of prey in running water (Coombs et al. 2001) since it filters out laminar background flow and therefore responds to local hydrodynamic stimuli about equally well under different flow conditions.

Compared to the amount of information that we have about peripheral lateral line function only little is known about the processing of lateral line information in the fish's brain. Obviously brainstem lateral line units are not particularly well adapted for the analysis of pure sine wave stimuli. This hypothesis is supported by the fact that most MON units are substantially less sensitive to vibrating sphere stimuli than are primary afferents (e.g., Paul and Roberts 1977; Caird 1978; Wubbels et al. 1993; Montgomery et al. 1996; Coombs et al. 1998), respond readily to the complex water motions generated by a moving object (Mogdans and Goenechea 2000) and have fairly large and complex receptive fields (Mogdans and Kröther 2001). Apparently,
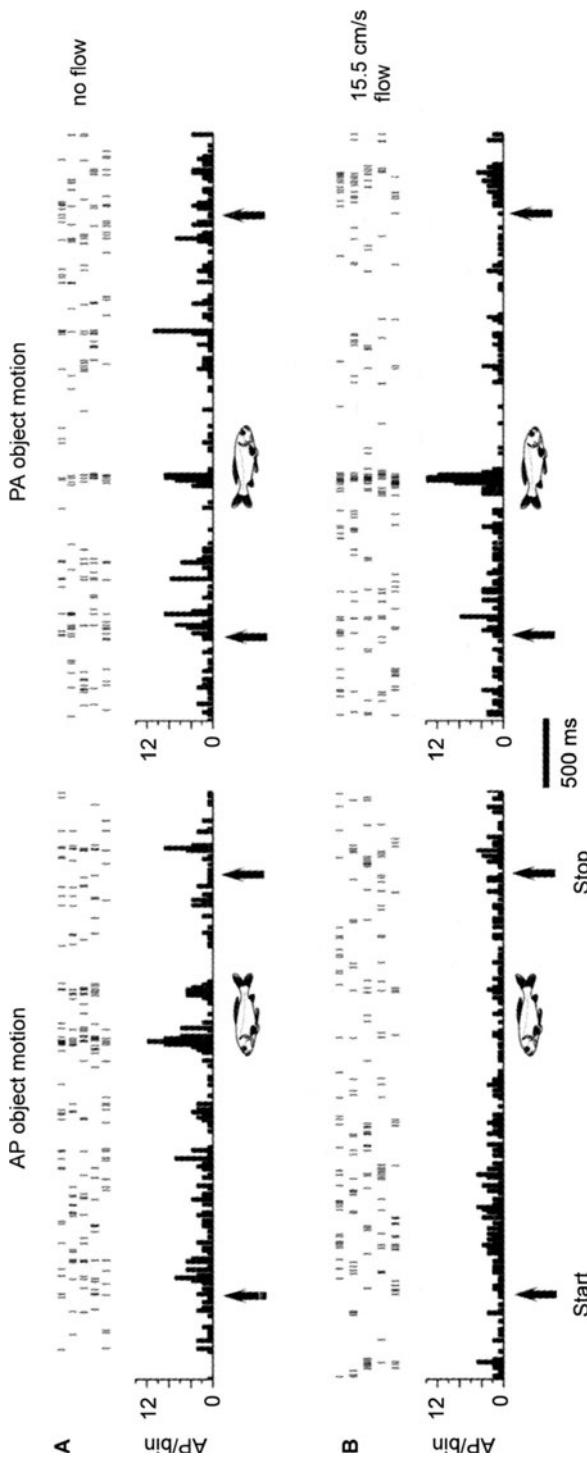
Fig. 10 Brainstem lateral line responses to a moving object (sphere, 8 mm diameter, speed 15 cm/s) in still and running water. Raster diagrams of the responses to three object presentations and the corresponding peri-stimulus-time histograms (binwidth 20 ms) of a goldfish MON unit are shown. Motion direction was from anterior to posterior (AP, left) or from posterior to anterior (PA, right). Fish symbols represent location, orientation and size of the fish relative to the path of the moving object. **A** Response to the moving object in still water. Compare responses with those shown in Fig. 4. **B** Response to the moving object in running water (10 cm/s flow velocity). Note that discharge rate was not changed in running water, i.e. this unit was flow-insensitive.

many MON units seem to be integrating inputs from a large number of neuromasts distributed widely across the lateral line periphery.

Among those MON units that respond to sine wave stimuli, a functional subdivision similar to that in the lateral line periphery can be found: whereas responses of type MI units are masked in running water, responses of type MII units are not affected (Kröther et al. 2002). This suggests that to a large degree information from superficial and canal neuromasts is processed separately in the fish brainstem. However, type MIII and type MIV units have response properties intermediate to those of type MI and type MII units. This may be due to convergence of inputs from the superficial and canal neuromast system at the first site of sensory integration in the ascending lateral line pathway.

Even though the biomechanical properties of lateral line neuromasts are well described (see also Janssen 2003, this volume), we still do not know much about lateral line function under natural hydrodynamic conditions. This is due to the fact that most of our knowledge comes from studies in which the lateral line was stimulated with sine wave stimuli applied in still water. It is unlikely that the lateral line has evolved for the analysis of sine wave stimuli since pure sine waves are extremely rare in nature. More likely the lateral line has evolved to analyze more complex water motions like those generated for instance by swimming animals like conspecifics, predators or prey. Thus, stimulating the lateral line with complex water motions that resemble those found in nature is important to understand its function. Likewise, only studies in which the lateral line is stimulated under background noise conditions, e.g., in a laminar or turbulent water flow, will reveal the functional limitations and evolutionary adaptations of this sensory system.

Acknowledgments

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Adaptation of the Rostral Ampullary Electrosense for Plankton Feeding by the Paddlefish

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ABSTRACT

The paddlefish paddle and its clusters of ampullary organs represent a novel evolutionary adaptation of the passive electrosense for zooplanktivorous feeding. The rostrum serves as an antenna extended in front of the fish to detect plankton encountered during continuous, ram-ventilating swimming. With its extensive population of highly sensitive ampullary electroreceptors, the paddlefish is ideally suited to utilize the rich planktonic resource, a diet of near microscopic organisms otherwise difficult to detect by visual predators in the turbid waters of the Mississippi River drainage. The zooplankton, primarily cladocerans, e.g., Daphnia, exhibit dipole DC and oscillating electric field potentials that are readily detected by the paddlefish. These environmental signals are transduced into neuronal responses in the primary afferent fibers of the anterior lateral line nerve. Each afferent fiber branches to innervate multiple ampullae within the peripheral clusters. The afferents terminate in the specialized electrosensory lobes of the hindbrain medulla, the large dorsal octavolateralis nuclei (DON). Electrosensory information is passed on to large pyramidal-like neurons in the DON that project robustly via brainstem tracts into the midbrain innervating the tectum mesencephalicus, the lateral mesencephalic nucleus, and the torus semicircularis. Electrosensory processing in the DON also involves descending input from both midbrain and cerebellar nuclei, in addition to commissural feedback from the contralateral DON. Direct innervation of the tectum from the DON is unique to the paddlefish and may reflect dominance of the electrosensory system in tectal orientation, a mechanism essential for prey localization and capture.

Key words: Paddlefish, *Polyodon spathula*, Ampullary Organs, Electrosense, Zooplankton

INTRODUCTION

Animals with specialized features provide attractive research topics for comparative evolutionary biologists. However, one such example, the paddlefish *Polyodon spathula*, has until recently escaped the attention of functional morphologists. This fish possesses a flat, greatly elongated

and eye-catching rostrum approximately one-third its length (Fig. 1). Once found throughout the Mississippi River basin of Midwest North America, the paddlefish has been widely misunderstood, as illustrated by its vernacular names *spoonbill cat* and *shovelfish*. These names imply that the rostrum forms a spatulate scoop to excavate food from the substrate although it is now well known that paddlefish feed entirely on zooplankton suspended in the water column. Our initial objective was to explain the adaptive significance of the elongate rostrum. No other fish possesses such a prominent forward extension of the cranium unless in association with the jaws and olfactory organs, neither of which extends into the paddlefish rostrum. We have shown that the rostrum is an electrosensory organ, exquisitely adapted as an antenna to detect and localize the weak electric fields of zooplankton (Wilkens et al. 1997). The paddlefish is the only fish know to have adopted the passive electrosense for plankton feeding.

ELECTROSENSORY STRATEGY FOR PLANKTON FEEDING

The paddlefish is one of two extant species in the family Polyodontidae, an ancient lineage of bony fish that along with sturgeons comprises the largely cartilaginous chondrostean order Acipenseriformes (Grande and Bemis 1991). *Polyodon spathula* is highly derived as the sole representative of the chondrosteans to adopt the planktivorous feeding mode, a strategy that utilizes the abundant planktonic biomass at the base of the food chain, the grazing daphnid crustaceans. Analogous with some of the marine zooplanktivores, e.g., the basking and whale sharks and manta ray, paddlefish grow large, historically reaching weights up to 70 kg. In terms of scale, the paddlefish and its cladoceran prey are proportionately equivalent to baleen whales and the euphausid krill on which they feed.

As a planktivore in the freshwater rivers and backwaters of the midwestern states, the paddlefish is ideally suited to harvest seasonal blooms of zooplankton. However, the waters of the Mississippi River and associated rivers and tributaries are turbid, a condition unfavorable for sight-based feeding on small, nearly microscopic prey, especially when large quantities are

Fig. 1 The paddlefish, *Polyodon spathula*, shown with its mouth open to an extent typical of ram-ventilating swimming. This picture of a small fish 16 cm long, shows the elongated rostrum, laterally-facing eye, and clusters of ampullary organs on the opercular flap.

required for the large and/or rapidly growing paddlefish. Freshwater fish that feed on zooplankton as visual predators are either small or they feed on plankton as juveniles, switching to larger prey as they grow. The paddlefish has successfully captured this resource niche by adopting an electrosensory mechanism of prey detection that is unaffected by turbidity.

There are several novel features that characterize the feeding strategy and life history of the paddlefish. First, these fish are obligate zooplanktivores throughout their life, i.e., they feed on plankton as larvae following depletion of the yolk sac, and continue as planktivores throughout their juvenile and adult life stages. Correspondingly, there is no wholesale metamorphic reorganization of the oral apparatus between the early stages and the adult fish. Rather, there is a shift in capture strategy in the juvenile stage that occurs with the development of comb-like rakers on the gill arches. Before gill rakers are present, small fish < 20 cm in length capture plankton individually (Rosen and Hales 1981) by selective particulate feeding. When gill rakers are present, fish begin to strain plankton from the water by a non-selective filtering mechanism, i.e., suspension feeding.

Second, paddlefish are ram ventilators and swim continuously throughout life (Burrgren and Bemis 1992). Suspension feeding is also basically a ram process whereby plankton are taken in with water drawn through the oral cavity while swimming. For both particulate and suspension feeding, paddlefish open their mouths widely to engulf plankton, an action that precludes generation of a high-velocity suction current for entrainment of prey. In suction feeding, characteristic of ambush predators, the mouth extends forward cone-shaped with a small orifice to generate high intake current velocity (Gerking 1994). As ram ventilators, paddlefish in principle need only to open their mouths to feed as they swim. However, particulate feeding typically involves a gulp-like motion of the mouth and buccal apparatus. For ram feeding in general, the electrosensory rostrum is ideally positioned in front of the mouth where it can detect the approach of plankton, much as prey is detected in front of an organism when using vision.

That the rostral electrosense is a specialized adaptation for plankton feeding under turbid conditions is reflected by the fact that vision, of limited use in this context, is poorly developed in the paddlefish. The eyes are relatively small and positioned at the base of the rostrum facing lateral and somewhat ventral to the fish. Even in clear water they would be poorly situated for detecting the approach of plankton. That vision is weak is illustrated by the fact that paddlefish maintained in large-volume culture tanks apparently do not see the opaque tank walls, bumping into them with a regularity that causes callous formation at the tip of the rostrum. Further, paddlefish exhibit little or no response to visual stimuli, either from above the water surface or from the side of glass-walled tanks.

BEHAVIORAL EVIDENCE FOR ELECTROSENSORY DETECTION OF PLANKTON

Our studies on the electrosensory basis of planktivory have focused entirely on particulate feeding in small juvenile fish (< 20 cm long) that capture plankton individually. To investigate feeding behavior, we created an artificial recirculating stream environment in which paddlefish swim in place in a 40-cm long viewing chamber with cross-sectional dimensions of 14× 14 cm.

The glass sides and floor of the chamber allow the swimming motions of the fish to be video taped from both lateral and ventral views. The propeller of a small trolling motor circulates the water, with a grill placed in front of the viewing chamber to restrict the fish and ensure laminar flow. Except for control feeding experiments under fluorescent room lighting, all experiments have been performed in the dark using infrared illumination of the tank and IR-sensitive video cameras. Plankton, either brineshrimp (*Artemia*) or water fleas (*Daphnia,* the natural prey), are added remotely downstream from the fish via tubing from outside the room and circulate freely into the viewing chamber and through the return plumbing. Although circulating plankton are viewed experimentally as approaching a stationary fish, this technique for analyzing prey capture is equivalent to natural feeding conditions where fish approach a relatively stationary plankter.

Particulate feeding by a swimming, ram-ventilating paddlefish involves a feeding strike in which the fish rapidly adjusts its swim path to intercept a plankter as it passes alongside the rostrum, in front of the mouth (Fig. 2). The onset of the strike (at $t = 0$) is indicated by promotion of the pectoral and pelvic fins and/or flexion of the trunk and occurs most often shortly after the plankton passes the mid length point of the rostrum (Russell et al. 2001). Promotion of the fins serves initially to slow the speed of the fish, a response more pronounced for distant prey requiring greater adjustments to the swim path. However, the rostrum, by virtue of its spatulate shape, places constraints on the swimming motions of the feeding strike. For plankton that pass lateral to the rostrum, the paddlefish needs only to flex its trunk to swing the rostrum in a yaw-like slicing motion toward the plankton, thereby bringing the mouth into position for capture. For plankton above or below the rostrum, strikes involve a combination of roll and yaw, motions that similarly allow the rostrum to slice through the water with least resistance. These motions are illustrated in the feeding strike of Figure 2 where the plankton was above and lateral to the rostrum, resulting in an acrobatic sequence of swimming motions ending with the fish nearly upside down.

The particulate feeding strike culminates with a gulp in which the fish engulfs a large volume of water containing the plankton. Characteristically, the gulp involves a wide gaping of the mouth, as illustrated in the final frame of Figure 2. The exaggerated dimensions of the strike aperture are further illustrated in Figure 3 where the fish has been induced to strike at electrodes used to simulate plankton. This view also illustrates the enormous expansion of the buccal cavity associated with the gulp, an expansion similar to that seen during filter feeding in larger fish (note the absence of gill rakers in this fish). There is a slight forward acceleration in swimming that accompanies the gulp (not shown) that follows the initial braking and turning motions that lead to capture. However, there is no detectable acceleration of the plankton toward the mouth, based on frame analysis at 30^{-s}. Thus, our initial analysis of plankton capture is consistent with a ram feeding mechanism.

Paddlefish feeding in the artificial stream has been studied extensively with regard to the distribution of captured plankton relative to the fish (Wilkens et al. 1997; 2001; Russell et al. 1999). More than 10,000 feeding events have been examined by off-line analysis of digitized video frames to show plankton locations as they would appear in front of the fish prior to
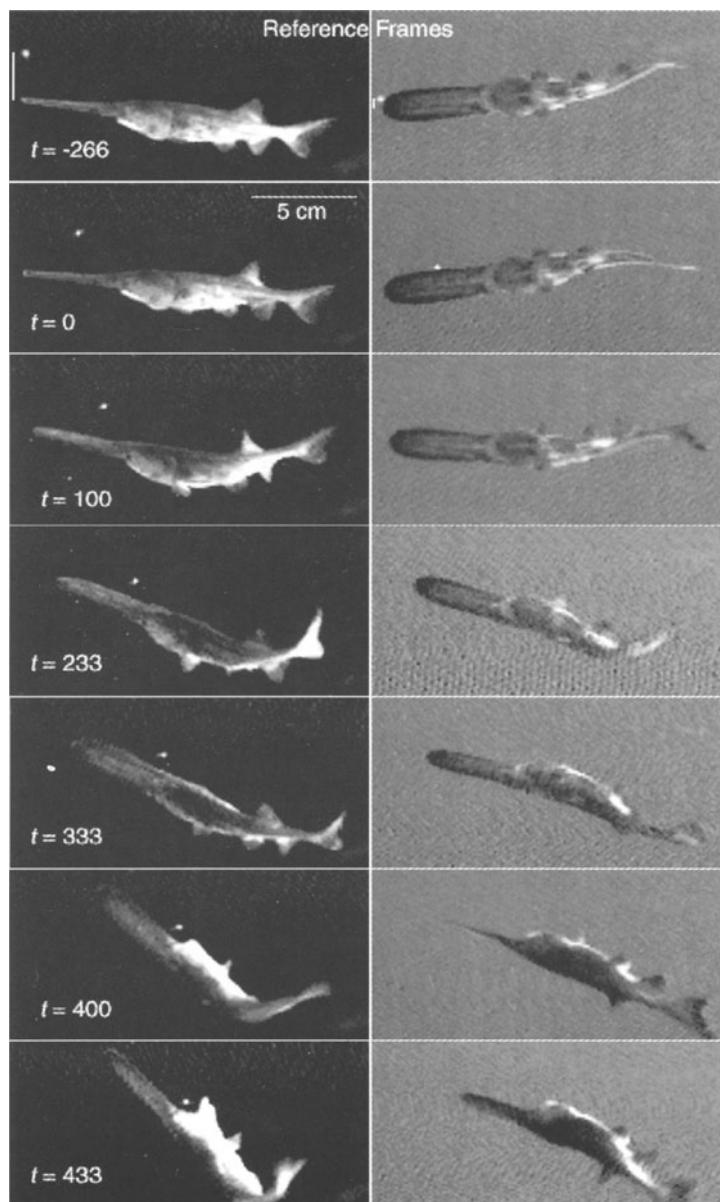
Fig. 2 Plankton feeding in juvenile paddlefish. A sequence of 7 video frames showing the fish swimming in place in the artificial stream with the approach of a single *Daphnia* (white spot). Lateral and ventral views are shown in the left and right columns, respectively. Zero time (in ms) in the capture sequence is shown in the second frame, when fin movements signal the initial response of the fish to the plankton. All captures were analyzed based on the location of plankton in a vertical reference plane at the rostrum tip, shown here at *t* = -266 ms. Vertical lines in the reference frames illustrate the vertical and horizontal distances used to quantify plankton locations. Frames have been computer-enhanced to increase contrast and highlight the plankton. The plankton is obscured in the ventral view after *t* = 0 by the lateral movement of the rostrum. Figure taken from Wilkens et al. 2002.

Fig. 3 Underwater photograph of a small paddlefish (16 cm long) striking at dipole electrodes simulating the electric field of a zooplankton.

capture, i.e., in register at the tip of the rostrum. Plankton distributions in this vertical reference plane are illustrated in a scatterplot showing pooled data from feeding experiments performed in the dark under IR illumination (Fig. 4A). As seen by inspection, plankton are captured in equal numbers above and below the rostrum, but with a greater lateral than vertical distribution, a pattern that mirrors the flattened shape of the rostrum. These data are essentially identical to control results obtained from fish feeding in the light (Wilkens et al. 1997). Clearly, paddlefish can detect and capture plankton without visual input, and all evidence suggests that they feed no more effectively in the light than they do in the dark.

Transformation of these data into radial distances from the center of the rostrum (Fig. 4B) shows a distribution pattern that is characteristic of feeding under a variety of experimental conditions. For these fish 17-22 cm in length and average rostrum width of 2 cm, most plankton captures were within 8-20 mm from the rostrum (56%), with the maximum number of captures at 13 mm. Relatively few plankton were captured close to the rostrum or at distances greater than 40 mm (4.3%), although paddlefish reliably capture plankton up to 90 mm from the rostrum.

Planktivorous feeding by the paddlefish in the dark was examined under several additional conditions each designed to eliminate detection of plankton using sensory modalities other than the electrosense (Wilkens et al. 2001). For example, to eliminate the possibility that paddlefish rely on near-field turbulence generated by the swimming appendages of plankton, or on chemical signals released by the plankters, we tested feeding by encapsulating each plankton in agarose gel. Individual *Daphnia* and/or *Artemia* were grasped by forceps and dipped twice to form a uniform gel coating before being introduced into the artificial stream. Microscopic examination revealed that plankton remained viable for up to 30 min following encapsulation. Analysis of capture distributions for 613 encapsulated *Daphnia* revealed no significant differences from the distributions of free-swimming plankton (Fig. 4B, inset), with maximum capture frequency again at 13 mm from the rostrum. Encapsulated plankton were then presented in feeding-choice format by adding an equal number of empty agarose particles of similar size to the recirculating water bath. Paddlefish easily discriminated between particles, capturing 368 encapsulated plankton but only 21 empty particles.

A

B
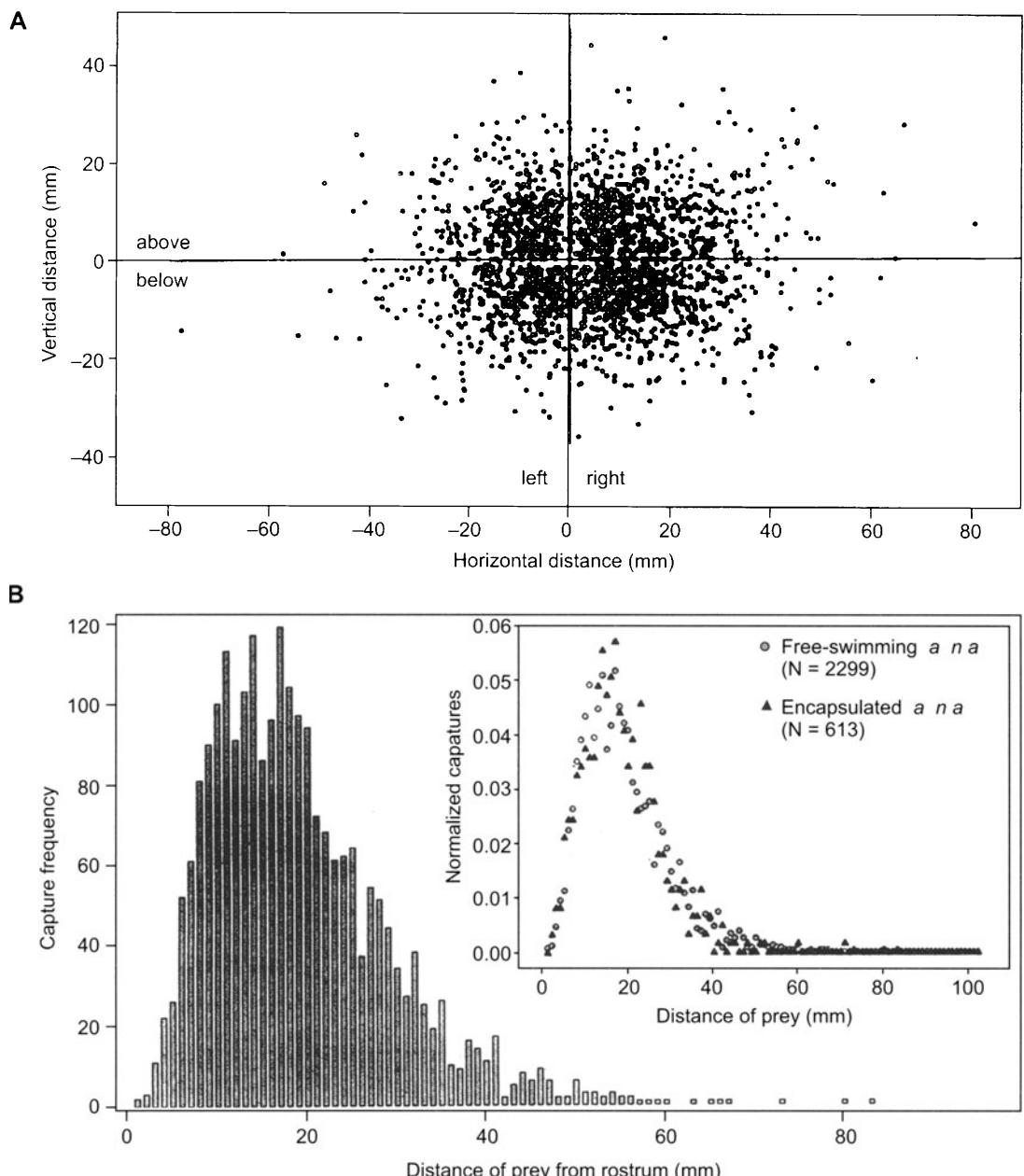
Fig. 4   Data excerpted from Wilkens et al. (2001) showing the distribution of captured zooplankton from feeding experiments in the artificial stream. A) Scatterplot marking the location of individual plankton prior to capture in a transverse reference plane at the tip of the rostrum. Data pooled from several experiments conducted in the dark (N=2299). B) Plankton captures (data from A) relative to their radial distance from the center of the rostrum. Inset shows histogram data normalized for comparison of capture distributions for free-swimming plankton and plankton encapsulated in agarose.

Finally, there was little if any effect on plankton feeding in experiments in which (1) a concentrated extract of plankton solution was added to the stream to mask potential chemical signatures of individual plankters (2) the nares were plugged to interfere with olfaction, and (3) turbulent flow was generated in the water entering the feeding chamber to mask the hydro-dynamic signals from swimming plankton. In each instance fish captured plankton with distribu-tions similar to those previously described (Wilkens et al. 2001). In fact, feeding was enhanced by the addition of plankton extract, as evident by an increase in the rate of capture.

Each of the feeding experiments described above supports the conclusion that paddlefish use their rostral electrosense to detect plankton by the elimination of other sensory options. This conclusion is further supported by experiments aimed more directly at the electrosensory system of the paddlefish. In one study (Russell et al. 1999), small paddlefish feeding in the artificial stream environment were subjected to uniform field electrical noise introduced by plate elec-trodes inserted at the upstream and downstream limits of the chamber. The effect of electrical noise on feeding varies with intensity. Low noise levels $0.1 \, \mu V \cdot cm^{-1}$ r.m.s. or less had no effect whereas higher noise levels of $5\text{-}20 \, \mu V \cdot cm^{-1}$ depressed feeding, as defined by a decrease in the spatial range of captures in vertical space about the rostrum. At $50 \, \mu V \cdot cm^{-1}$ r.m.s. noise paddle-fish stopped feeding entirely. Interestingly, however, at intermediate noise levels (0.5-0.7 $\mu V \cdot cm^{-1}$) plankton feeding was enhanced, as measured by a broadening of the spatial range of plankton detection and capture. This non linear effect of noise is referred to as "stochastic reso-nance", a phenomenon in which empirically-determined optimal noise is seen to improve the detection of weak signals (Wiesenfeld and Moss 1995). That electrical noise can both enhance and depress feeding provides direct support for an electrosensory mechanism of sensory detec-tion of plankton.

A final behavioral demonstration of electrosensory-based feeding was obtained by inducing feeding strikes at dipole electrodes through which weak electrical currents were passed to simu-late the weak electric fields of plankton (Wilkens et al. 1997; Wojtenek et al. 2001b). Sinusoidal currents over a range of intensities and frequency were presented in random sequence. Feeding strikes at these artificial signals, such as illustrated in Figure 3, were elicited in the dark and recorded on videotape using infrared illumination. Fish swimming in the vicinity of the elec-trodes swerve to investigate the signal and strike one or more times in a fashion equivalent to the feeding strikes aimed at live plankton, i.e., a ram-feeding gulp as described above. Dipole strikes were elicited predominantly at low stimulus frequencies in the range of 5-15 Hz, with maximum strike frequency at 5 Hz. Strike frequency also varied with current intensity, with maximum numbers of strikes at $0.25 \, \mu A$ p-p and reduced strike frequencies at higher and lower intensities. At the water conductivity used in these experiments ($4 \, mS \cdot cm^{-2}$), this intensity is equivalent to a field potential of $62.5 \, \mu V \cdot cm^{-1}$ at the electrode tips. The frequency-intensity response charac-teristics of the dipole stimulus mimic the electric fields of planktonic prey in the environment. For example, *Daphnia* produce oscillating potentials with maximum power in the range of 3-7 Hz (see below) and exhibit surface potentials of $0.3\text{-}1.0 \, \mu V \cdot cm^{-1}$ that attenuate rapidly with dis-tance. Thus, there is good agreement between the artificial and natural electrical signals that trigger feeding strikes, results that strongly support the electrosensory basis of plankton feeding by the paddlefish.

ELECTRICAL SIGNALS IN THE PADDLEFISH ENVIRONMENT

The aquatic environment is inherently noisy, with electrical signals originating from a variety of physical, electrochemical, and biological sources (Peters and Bretschneider 1972; Kalmijn 1974; 1988). In characterizing the ampullae of Lorenzini, the first evidence for paddlefish electrosensitivity, Jørgensen et al. (1972) also noted that paddlefish are startled by the galvanic potentials of an iron tube. We have extended this observation by quantifying the sensitivity and avoidance behavior of paddlefish to a 2.5 cm diameter aluminum rod (Gurgens et al. 2000). Fish detect and avoid this obstacle without fail, turning away at an average distance of 25 cm, in contrast to a plastic obstacle that they do not detect and with which they frequently collide. Paddlefish in their natural environment are also reported to avoid metal obstacles, e.g., the steel gates of locks and dams (Southhall and Hubert 1984), but their sensitivity to more natural sources of electric fields, e.g., ore deposits, river currents and other geomagnetic phenomena, is unknown. It was speculated that as plankton feeders, paddlefish might sense the electrochemical gradients associated with swarms of plankton (Kalmijn 1974). Although we now know that juvenile paddlefish detect the electrical potentials of plankton, the sensitivity of large filter-feeding paddlefish has not been investigated. However, sensitivity to electrochemical gradients would need to be distinguished from olfactory cues associated with plankton swarms.

Many electrosensory fish also generate electric potentials, electric organ discharges (EODs) that they use to "actively" sense their electrical environment (Nelson and MacIver 1999; von der Emde 1999; Keller, this volume). By measuring distortions of their EOD current fields, weakly electric fish can detect not only external field potentials, but also the capacitative and resistive properties of an object. Thus, they are able to actively sense both animate and inanimate objects (von der Emde 2001). For passive electrosensory fish, e.g., elasmobranchs and the paddlefish, sensitivity relies on the presence of an externally generated electric field. The use of the passive electrosense for locating animate objects in the environment is illustrated by the classic demonstrations of prey detection by the shark (Kalmijn 1971) and mate detection by the stingray (Tricas et al. 1995; Tricas and Sisneros, this volume), in both cases with the prey or potential mate buried in the sand.

Bioelectric fields for a number of vertebrate (fish) and invertebrate organisms have been documented (Keller, this volume). For marine organisms, bony fish have DC fields of $\sim$500 $\mu V \cdot cm^{-1}$ whereas skates and rays generate fields an order of magnitude weaker (Kalmijn 1972), the latter value confirmed by more recent recordings from the stingray (Tricas et al. 1995; Tricas and Sisneros, this volume). These steady offset potentials are modulated by low-frequency "ripples", bioelectric currents modulated in turn by respiratory activity; muscle potentials were distinguished separately as small ($< 10 \mu V \cdot cm^{-1}$), high-frequency spikes. Marine invertebrates in general exhibited small potentials ($10 \mu V \cdot cm^{-1}$) except for mollusks ($100 \mu V \cdot cm^{-1}$). Curiously, crabs and shrimps generated relatively small potentials ($50 \mu V \cdot cm^{-1}$) unless injured, in which case potentials exceeded $1 mV \cdot cm^{-1}$ (Kalmijn 1972). All potentials were measured approximately 1 mm from the surface of the animal.

In the freshwater environment the bioelectric field potentials of organisms are much greater. Peters and Bretschneider (1972) recorded $1-2$ mV·cm^{-1} potentials from several species of fish that they characterize as DC dipole fields with superimposed respiratory fluctuations. DC potentials of 100-$200\,\mu$V·cm^{-1} were recorded from tadpoles and insect larvae, again modulated by respiratory activity. These potentials were all recorded 5 mm above the surface of the organism, a distance at which a potential recorded at 1 mm, as in the marine examples, would be significantly attenuated.

Overall, aquatic organisms give rise to DC dipole fields modulated by oscillating potentials about 10% of the amplitude of the DC potentials. The examples cited above all refer to macroscopic organisms, with no plankton measurements available for comparison. The suggestion that paddlefish might sense electrochemical gradients from clouds of plankton may reflect the fact that crustaceans in general exhibited small field potentials that, extrapolated to the small size of crustacean zooplankton, might be imperceptibly small. This may in fact be true for marine plankton. However, our recordings have shown that *Daphnia* (and, in freshwater, the brineshrimp *Artemia*) generate substantial dipole electric fields.

To record planktonic potentials we developed drift-free Ag-AgCl electrodes with which we could record DC-coupled signals at a sensitivity of $10-20\,\mu$V $\times$ div^{-1}. *Daphnia* were tethered to a fine glass filament manipulated via a pen motor shaft and swept slowly past the tip of the recording electrode; a reference electrode was placed $15-20$ cm away. The mounting filament could be rotated so that different plankton surfaces were proximal to the electrode tip, i.e., dorsal, caudal, ventral, anterior, etc. Spike-like potentials were recorded as the plankton first approached and then retreated from the electrode at constant velocity. Alternately, a plankton was held stationary adjacent to the electrode to record oscillating potentials. Water conductivity was adjusted to $500\,\mu$S·cm^{-1}.

Potentials representative of the largest signals recorded from *Daphnia* are presented in Figure 5. With the ventral surface proximal to the electrode a positive spike was evident, whereas the antero-dorsal surfaces produced negative spikes (Fig. 5A). For intermediate anterior and posterior surfaces a smaller biphasic signal was recorded. When held stationary, oscillating potentials were recorded with amplitudes characteristic for various appendage movements. As illustrated in Figure 5B, antennal potentials were readily distinguished from those of the smaller thoracic filtering appendages. Fourier analysis of the signals from tethered *Daphnia* revealed frequency maxima of 3 and 7 Hz.

These results indicate that freshwater plankton generate sizeable electric signals. Although somewhat complex, *Daphnia* potentials generally describe a dipole DC field approaching 1 mV·cm^{-1} at the immediate surface of the animal, modulated by smaller $30-100$ mV amplitude oscillations representing muscle potentials from the appendages. Potentials fall off rapidly with distance from the plankton, as indicated by the rapid decline in the recordings illustrated in Figure 5A. Each trace represents a 1-cm excursion of the plankton, with closest proximity to the electrode at the midpoint of the trace. Plankton potentials are also variable, with different polarities and (smaller) amplitudes than those illustrated here. The rapid decline in planktonic field potentials with distance, approximated by a power function (-2), indicates that the
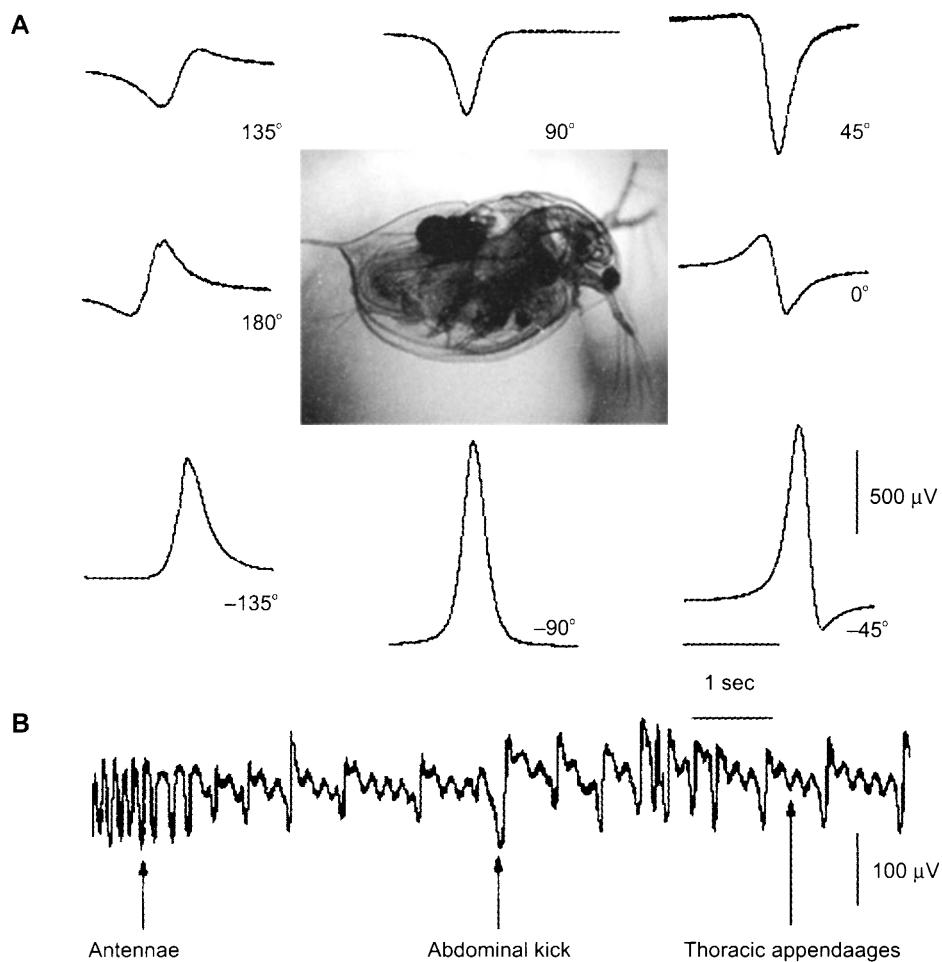
Fig. 5 Plankton potentials from a *Daphnia* fixed to the tip of a 26-cm arm and swept close to the recording electrode in a 6-cm arc centered on the plankton. With the plankton at either end of the arc the potential was defined as 0 mV. (A) Positive, negative, and biphasic peaks were recorded as the plankton passed the electrode, with potentials varying as the *Daphnia* was rotated about its sagittal plane at 45° intervals. (B) Higher gain recordings of the oscillating potentials associated with various appendage movements recorded from a stationary plankton. Control recordings with a fine agarose-coated screen placed between the plankton and electrode tip insured that these potential waveforms were not movement artifacts.

threshold sensitivity of the paddlefish electroreceptors is extremely low. Estimates of field potential amplitude at maximum detection/capture distances up to 9–10 cm are around 0.1 $\mu V \cdot cm^{-1}$, a value in close agreement with the stochastic resonance thresholds for noise-enhanced feeding (0.2 $\mu V \cdot cm^{-1}$, Russell et al. 1999). *Daphnia* swarms also produce electrical signals in the form of low-frequency (to 20 Hz) noise, detectable experimentally (0.3 μV r.m.s.) at distances up to 5 cm (Russell et al. 1999).

FUNCTIONAL MORPHOLOGY OF THE ROSTRAL ELECTROSENSE

The phylogenetic distribution of electroreceptive ampullary organs includes most major taxa of aquatic lower vertebrates inclusive of the chondrichthyans, lobe-finned fish plus aquatic amphibians, and non-neopterygian ray-finned fishes, the polypterids, sturgeons and paddlefish. Paddlefish ampullary organs are the most numerous of any fish, with estimates up to 75,000 (Nachtrieb 1910), owing to their extensive distribution over the elongated rostrum (Fig. 6) and opercular flaps (Fig. 1). These "primitive pores" or "sensory pits" were long suspected as being sensory organs but their exact function remained the subject of considerable debate (Kistler
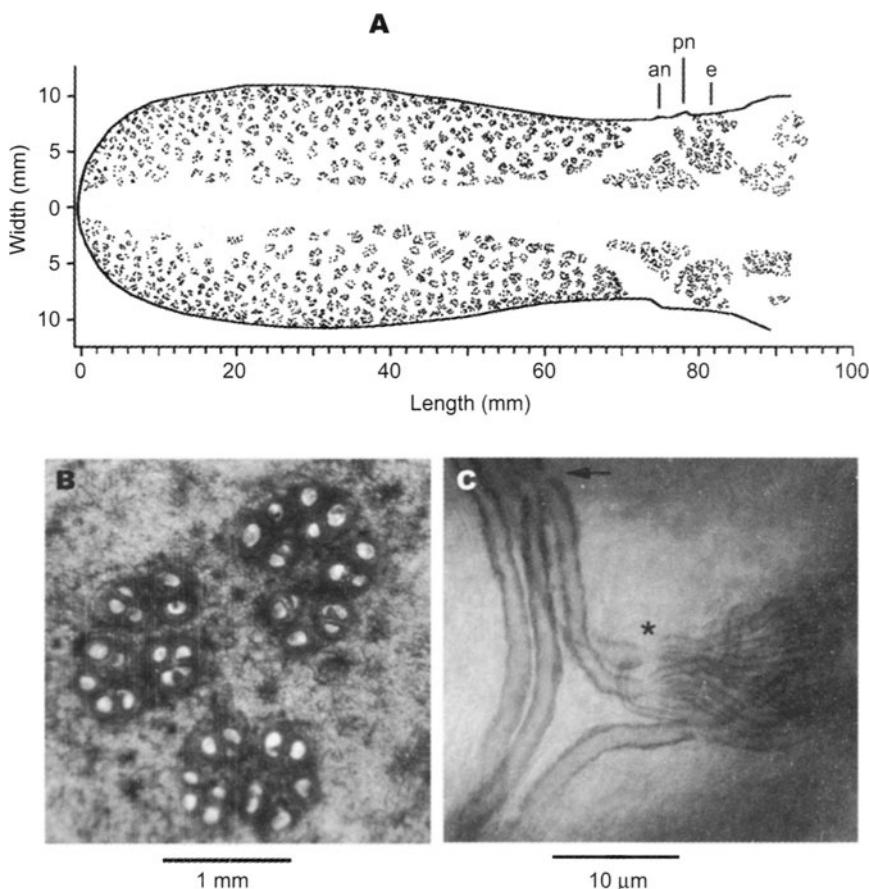
Fig. 6 Ampullary organs on the paddlefish rostrum and head. (A) Dorsal view of ampullae clusters forming lateral fields on the rostrum, above the anterior (an) and posterior (pn) nares, the eyes (e), and onto the cranium. A montage of digital photographs and NIH Image software were used to map the ampullae (experiments performed by Lukas Voigt and Winfried Wojtenek). (B) Close up view of 3 clusters illuminated from below. (C) A fine branch of the ALLN stained with Sudan Black that curves over the edge of a cluster of 8 ampullae, giving off 3 primary afferent axons that project into the center of the cluster. Myelin nodes are visible at the arrow and where the axons branch into finer process (*). Panel C taken from Figure 7D, Wilkens et al. 2003.

1906; Nachtrieb 1910; Norris 1923). Definitive identification of the ampullae as electroreceptors was based on their structural similarity to the ampullae of Lorenzini of elasmobranchs (Jørgensen et al. 1972). Together with those on the cranium and lower jaw, paddlefish ampullae constitute a spatially distributed sensory organ of the head that encompasses more than half the length of the fish. This emphasis on ampullary organs was interpreted as compensating for the poorly developed visual and chemoreceptive sensory modalities (Bemis et al. 1997), a prediction borne out by our studies of electrosensory-mediated feeding and avoidance behaviors. The putative papillary electroreceptors of the snipe eel, a mesopelagic fish, are believed similarly to compensate for loss of, or greatly reduced, vision in these fish (Meyer-Rochow 1978).

Paddlefish ampullae, as described by Jørgensen et al. (1972) extend from surface pores into a pear-shaped lumen up to 120 mm in diameter and 250 mm deep. The lumen is filled with a jelly-like substance and lined along its basal surface by a sensory epithelium of ciliated receptors interspersed with supporting cells. Each receptor cell makes numerous synaptic connections with the branching terminals of primary afferent neurons that make up the anterior lateral line nerve (ALLN). The ALLN also contains mechanosensory afferents innervating the neuromasts of the cephalic lateral line.

Ampullae are found in clusters of 5–20 (Fig. 6B) that contribute to discrete fields of ampullary organs, e.g., on the lateral flaps of the rostrum and patches above the nares, eyes and lateral surface of the cranium (Fig. 6A). Each field is associated with a major ramus of the ALLN. Ampulla clusters are absent from the midline of the rostrum and head, areas underlain by sheets of median rostral bones. Correspondingly, clusters are found only in the soft spaces between the network of stellate bones that make up the lateral flaps of the rostrum, and on the fleshy opercula and skin of the lower jaw. Each ALLN ramus anastamoses into a network of fine nerves that spread out to innervate the ampullary clusters. Our initial neuroanatomical results show fiber bundles containing 3-5 myelinated axons innervating a single cluster (Fig. 6C), with a ratio of approximately one afferent neuron per three ampullae (Wilkens et al. 2003). Each afferent neuron subsequently branches into finer terminal dendrites within the cluster (Fig. 6C).

The large, paired ALLN nerves course posteriorly alongside the brain, entering the hindbrain alongside the octaval nerve. The cell bodies of the primary afferents are located in the root ganglion of the ALLN, a swelling of the nerve at the point where it angles medially toward the brain. Nerve fibers in the ALLN segregate into dorsal and ventral roots as they enter the medulla (New and Bodznick 1985). The dorsal root is made up exclusively of electrosensory units that terminate in the dorsal octavolateralis nucleus (DON), a prominent ridge that extends the length of the medulla above the cerebellar crest (Fig. 7A). The ventral root is exclusively mechanosensory, its fibers terminating in the medial octavolateralis nucleus (MON). As this innervation pattern shows, the DON is specialized as an electrosensory nucleus, a feature shared with other electrosensory fish. However, the DON is proportionately much larger in the paddlefish than in other species, e.g., in comparison with sturgeon (New and Bodznick 1985), again reflecting the relative importance of the electrosense in the paddlefish.

The general electrosensory circuit in the paddlefish brain has been outlined in recent experiments using biotinylated dextran amines (BDA: 3000 MW, Molecular Probes) to stain ascending and descending pathways connecting with the DON (Hofmann et al. 2002; Wilkens et al. 2003). Fine needles coated with dextran crystals were inserted into the DON and other areas of the brain after which fish were recovered and returned to their holding tanks for 3 days to allow uptake and transport of the tracer. Afterward, fish were killed by transcardial perfusion with buffered 4% paraformaldehyde and their brains removed, embedded in gelatin and sectioned with a vibratome. Sections (100 mm thick) were rinsed in buffer, incubated in avidin-biotin solution (ABC Kit, Vectastain) and stained with diaminobenzidine to visualize neurons incorporating the tracer.

Tracer injections in the upper pars dorsalis layer of the DON (DONd) revealed the massive input from the retrogradely stained ipsilateral dorsal root afferents. In addition, large pyramidal-like neurons were stained prominently in the DON. These neurons with cell bodies in the intermediate layer (DONi), were stained anterogradely via large dendrites that extend up into the DONd. These neurons ascend into the midbrain via brainstem tracts in the contralateral lateral lemniscus and project to the torus semicircularis (TS), the lateral mesencephalic nucleus (lmn), and the mesencephalic tectum (TM), the latter projection being especially well developed (Fig. 7E).

The DON also receives descending feedback input from hindbrain nuclei, as demonstrated by retrograde staining of axons terminating in or near the site of DON injections. Smaller pyramidal-like cells that project from the contralateral DONi (Fig. 7B) mediate commissural feedback. Retrogradely labeled cells were also found in the nucleus preeminentialis (NPe, Fig. 7C) and in the cerebellar auricles (Cer, Fig. 7D). The NPe inputs to the DON represent indirect feedback from the tectum, as revealed by tectal injections that project to the NPe. NPe feedback also tracks through the cerebellar auricles as shown by tracer injections in the auricles that labeled numerous inputs from the ipsilateral NPe. Granule cells stained anterogradely in the cerebellar auricles project in turn to the DON with parallel fibers running the length of the cerebellar crest (ventral layer of the DON) to contact ventral dendrites of the principal pyramidal-like neurons. These ascending and descending electrosensory pathways are summarized diagrammatically in Figure 7F.

The feedback loops to the paddlefish DON indicate that this large primary integrating center for the electrosense receives considerable descending input from the midbrain, as is also true for other electrosensory fishes (Nieuwenhuys 1998), and suggests that complex information processing at this level is a general feature. Significant differences exist, however, for the ascending pathways in the paddlefish brain where second-order electrosensory projections from the DON to the midbrain are more robust (Hofmann et al. 2002). Here the DON projects directly to the tectum, torus semicircularis, and lateral mesencephalic nucleus. As the TS and lmn also project to the tectum, the TM receives both direct and indirect input from the DON. In elasmobranches second-order neurons project only to the TM and lmn whereas in teleosts they project only to the TS. These differences suggest that electrosensory inputs to midbrain nuclei of the paddlefish, the tectum in particular, are indicative of a greater role for the
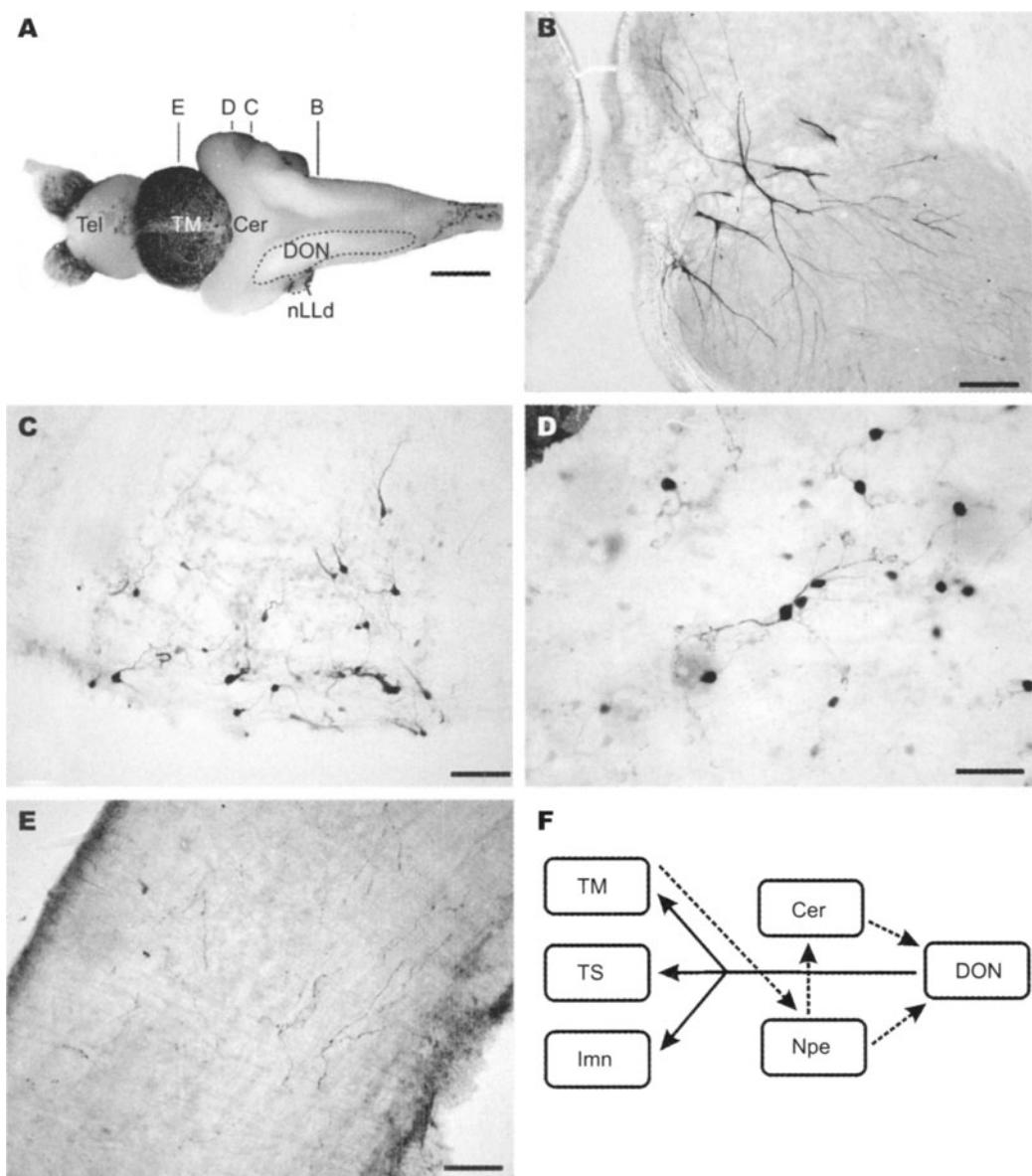
Fig. 7 (A) Dorsal view of the brain of the paddlefish (scale bar 3 mm). (B-E) Cross sections through the brain at levels indicated in (A. B-D) Unilateral tracer application into the dorsal octavolateral nucleus (DON) retrogradely filled cells in the contralateral DON (B, scale bar 200 µm), in the nucleus preeminentialis (C, scale bar 200 µm), and in the cerebellar auricles (D, scale bar 100µm). (E) A strong projection of cells in the DON reaches the mesencephalic tectum (scale bar 200 µm). (F) Schematic drawing of the ascending (solid lines) and descending (dashed lines) projections of the electrosensory system in the paddlefish. Abbreviations: BO: bulbus olfactorius; Cer: cerebellum; DON: dorsal octavolateral nucleus; lmn: lateral mesencephalic nucleus; nLLd: dorsal root of the lateral line nerve; NPe: nucleus preeminentialis; Tel: telencephalon; TM: mesencephalic tectum; TS: torus semicircularis. Figure taken from Wilkens et al. 2003.

electrosense in controlling the orientation functions normally assigned to the tectum (Dicke and Roth 1994; Vanegas et al. 1984). Therefore, we predict that topographic representation of electrical space in the mesencephalic tectum will be integral to the capture of plankton by the paddlefish.

The electrical properties of paddlefish ampullary organs parallel their anatomical similarity to the ampullae of Lorenzini (Jørgensen et al. 1972). As previously described (Wilkens et al. 1997), paddlefish ampullae are low-frequency receptors (1–20 Hz) that are excited by cathodal stimuli and inhibited anodally, equivalent to the sensitivity characteristics of other ampullary receptors (Murray 1962; Szamier and Bennett 1980; Bullock 1974; Kalmijn 1988).

We recorded activity from primary afferent neurons in the ALLN, initially from their axons in the fine rostral nerve branches, but more often from the larger cell bodies in the root ganglion that give stable, higher signal-to-noise recordings. Fish were anesthetized with MS222 (1:10,000, Sigma Chemical Co.), mounted in an experimental chamber with oxygenated water supplied to the gills, and the brain and ALLN were exposed by removal of the overlying chondrocranium. Fish were then paralyzed with curare, the wound site treated with Lidocaine, and the anesthetic washed out according to procedures approved by the institutional IACUC committee. Recordings were made with conventional tungsten or glass pipette microelectrodes. A stimulating dipole electrode with a tip separation of 1 cm was used to identify receptive fields and to characterize frequency and polarity sensitivity. In addition, *Daphnia* and *Artemia* were cemented to a fine glass filament (VetBond, 3M Co.) and attached as above to a pen motor shaft for manipulation to characterize ampullary sensitivity to more natural stimuli, i.e., their planktonic prey.

Paddlefish electroreceptors respond vigorously to nearby plankton, to both the DC dipole and low-frequency oscillating field potentials. Figure 8A illustrates high-frequency bursts of spikes (to 300 Hz) in synchrony with the antennal potentials of *Daphnia*. Here the plankter was held 2 mm above the surface of the rostrum and centered over the receptive field, with an electrode positioned nearby to monitor the planktonic signals. The bursts are synchronized with the negative (cathodal) antennal peaks although close examination of the traces shows phase variability. This is due to the difference in receptive field orientation of the receptor relative to that of the electrode in the pickup of the planktonic signal. Indeed, a slight rotation of the plankton will alter both the electrical recording and receptor response, which illustrates the variability in the field potential surrounding the plankton, as illustrated in Figure 5A.

In the same experiment, a *Daphnia* was swept slowly across the rostral receptive field at $4 \text{ mm} \times \text{s}^{-1}$ using a continuous triangular driving waveform (Fig. 8B). This type of stimulus is more representative of the dynamic signal a plankter would deliver to an approaching fish. At close range the larger dipole potential of the plankton elicits a strong response consisting of both excitatory and inhibitory components. As before, the signals picked up by the receptors and electrode are not equivalent and therefore the responses are not perfectly in phase. Nevertheless the anodal pulse of the plankton dipole potential corresponds with the inhibitory pause in the caudally-directed stimulus, followed by a cathodal spike burst. A reverse sequence of burst-inhibition-burst is elicited by the opposite rostrally-directed stimulus. This directional
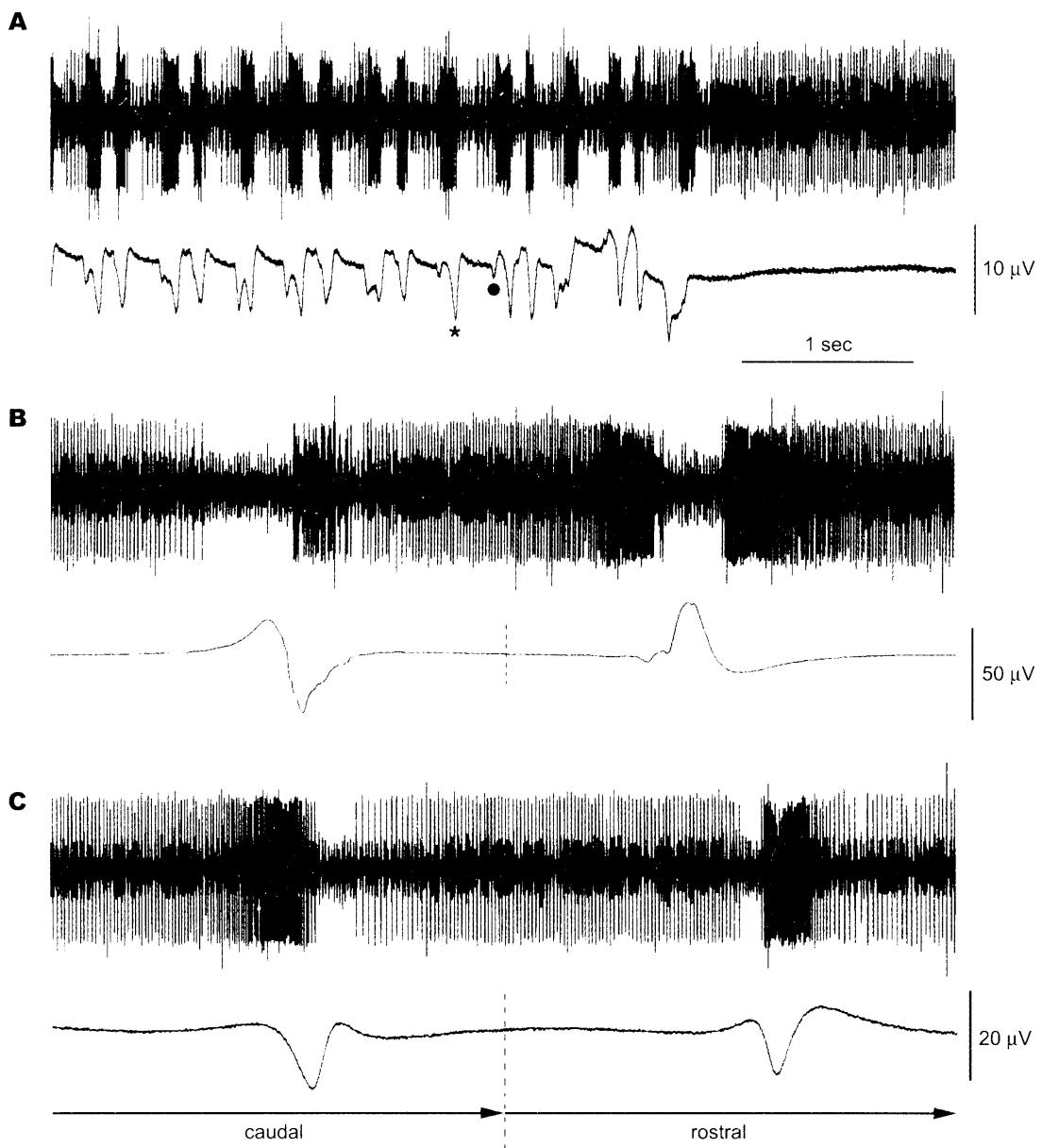
Fig. 8 Activity from an electrosensory primary afferent (large spike) in the dorsal ALLN root ganglion in response to the electrical signals of *Daphnia* (A, B) and a pellet of fish chow (C). The small spike is not identified. An electrode adjacent to the receptive field of the afferent was used to record plankton and pellet potentials (lower traces). Spike oscillations triggered by a stationary plankton (A) are in response to large antennal (*) and smaller thoracic appendage (•) potentials. The time base is the same for all traces. Arrows (B, C) indicate movement of the stimulus from the tip of the rostrum toward the eyes (caudal) and vice versa (rostral), with the vertical dashed lines indicating the approximate reversal point. D. Russell assisted with these experiments.

sensitivity in the response of the primary afferent reflects the asymmetry of the planktonic dipolar field potential, as illustrated earlier (Fig. 5A), with an excitatory or inhibitory response dependent on the orientation of the dipole field of an approaching plankter. Similar results in histogram and raster plot form have been presented elsewhere (Wojtenek et al. 2001a; Wilkens et al. 2003). Also, we have performed control experiments to insure that these responses are due to the plankton. Both the field potential and the primary afferent response are eliminated if the plankter is removed, i.e., there is no response to the supporting glass filament. Substitution of a ball of wax similar in size to the plankton also fails to elicit a response.

A similar result can be elicited by substituting a pellet of fish chow for the *Daphnia* and sweeping it over the receptive field (Fig. 8C). Again, the response is asymmetric, with mirror image excitation-inhibition profiles depending on the direction of the stimulus movement. Two points are of interest here. First, an inanimate food pellet produces a field potential easily measurable by the ampullary receptors, consistent with the observation that paddlefish feed effectively on pelleted food in the dark, as observed by infrared videography. Capture motions are similar to those for live plankton. Second, as with plankton, the fish pellet generates a response pattern dependent on stimulus dynamics, i.e., the direction of movement. One explanation for this difference is that the dipole potentials of both pellet and plankton create distinct stimulus patterns as they drift over the receptive field in different directions, a difference that is reflected qualitatively in our recorded plankton/pellet potentials. A second possibility is that paddlefish electroreceptors, in addition to their low-threshold sensitivity, are directionally sensitive. If this is the case, we speculate that it arises due to a difference in relative synaptic input from the ampullary receptors innervated within a cluster. In this event, signal processing would begin in the periphery prior to the arrival of sensory input to the brain.

Nevertheless, these results demonstrate that paddlefish ampullary organs can detect subtle electrical features of their plankton prey and therefore provide a wealth of information to the brain of the fish. How much of this information is important is not known. For example, it is unlikely that plankton orientation, and its attending sensory response, is important for capture. It will be interesting to learn how the paddlefish brain extracts relevant information from its electrosensory organs and how it processes this information to elicit accurate strikes resulting in plankton capture.

Acknowledgements

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Ecological Functions and Adaptations of the Elasmobranch Electrosense

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ABSTRACT

Sharks and rays have a long evolutionary history as major predators in marine ecosystems, but the biological functions and selective pressures that shape the evolution of their ampullary electrosensory system are poorly known. The ampulla of Lorenzini is the functional electrosensory unit that consists of a small subdermal ampulla and a canal that projects to a surface pore on the head or pectoral fins. The sensory epithelium of the ampulla wall detects differences between the potential at the skin pore and internal potential of the animal, and stimulates neural transmission of information about the physical features of an external field to the brain. Natural weak electric stimuli include polar fields from bioelectric sources and induced fields from physical sources in the environment. Neurophysiological studies show that the ampullary electrosense responds to electric field gradients as low as 20 nV/cm, and behavioral studies show responses to gradients of 1-5 nV/cm. Elasmobranch fishes show behavioral responses to bioelectric stimuli produced by natural prey, mates, consexuals and potential predators. Numerous models exist for electrosensory navigation, but they remain to be rigorously tested. Recent work shows age-dependent changes in the response properties of the electrosense among embryo, juvenile and adult stages and are proposed to reflect ontogenetic adaptations to their changing environments. In addition, the electrosense response properties are seasonally modified by the periodic expression of gonadal steroids and may serve important modulation of sensory function during reproductive behaviors. Future work should continue to investigate different biological contexts in which the electrosense is used by elasmobranch fishes, and to test the selective forces that may have shaped the evolution of this remarkable sensory system.

Key words: Ampulla of Lorenzini, Behavior, Elasmobranch, Electroreception, Neuroecology, Ray, Shark, Sensory Biology

INTRODUCTION

The living elasmobranch fishes (sharks and rays) share with their ancestors many morphological characteristics that directly control their behavioral capabilities and influence their ecology.

Most sharks have a large mouth with well-developed dentition, a torpedo shaped body and paired fins like their predecessors that lived more than 400 million years ago. Thus, the sharks have evolved into highly adapted carnivores that are capable of rapid swimming or ambush movements to capture their prey. Similarly, the derived skates and rays (collectively known as the batoids) have retained the features of their ancestors of 100 million years ago that include ventral gill slits, expanded pectoral fins and a dorsally flattened body. This highly successful group has since diverged to primarily exploit the two-dimensional worlds associated with benthic habitats.

Sharks and rays also share with their ancestors several exquisite sensory systems that have evolved in response to numerous selective pressures within their natural environment. Selective forces that may shape the form and function of sensory systems include the efficient capture of prey that increases feeding success, and the capacity to detect and avoid predators during different phases of development. The ability to locate potential mates, engage in courtship behaviors and successfully mate may directly enhance reproductive success. In addition, there can be great fitness advantages for sensory systems that provide spatial details of their home range, or provide orientation cues during long migrations.

Of the many sensory systems possessed by sharks and rays, the ampullary electroreceptors are the most unique and enigmatic in terms of function in the natural lives of these animals. While much is known about the structure, proximate mechanisms of sensory transduction and encoding of weak electric stimuli by the ampullary receptors, only recently has experimental work begun to address a wide range of biological functions for the electrosense in natural settings. In this chapter we first review the gross anatomy of the ampullary system of marine elasmobranch fishes, the receptors, and the neurophysiological responses of electrosensory neurons. We then present a summary of the experimental and theoretical functions for the electrosense of the sharks and rays within some of the biological contexts in which they have evolved. Other reviews of the electrosensory system of marine and freshwater elasmobranchs, and teleosts can be found in Bullock and Heiligenberg (1986), Zakon (1988) and New and Tricas (1998).

MORPHOLOGY AND PHYSIOLOGY OF THE AMPULLARY SYSTEM

The functional unit of the electroreceptor system is a highly specialized structure known as the ampulla of Lorenzini. Each ampulla consists of small continuous alveolar sacs that are positioned around the base of a single canal (Fig. 1A). The canal is approximately 1 mm in diameter and extends from the subdermal ampulla through the dermis and terminates as a small pore on the skin. The wall of the ampulla contains the sensory epithelium that is innervated by primary afferent sensory neurons. Individual ampullae are arranged in 3-4 clusters on each side of the body with the canals radiating outwards to their surface pores of the head (Fig. 1B). Canals usually project in many directions from each cluster and their pores are distributed widely over the surface of the head, and in the skates and rays extend on to the expanded pectoral fins (Fig. 1C).
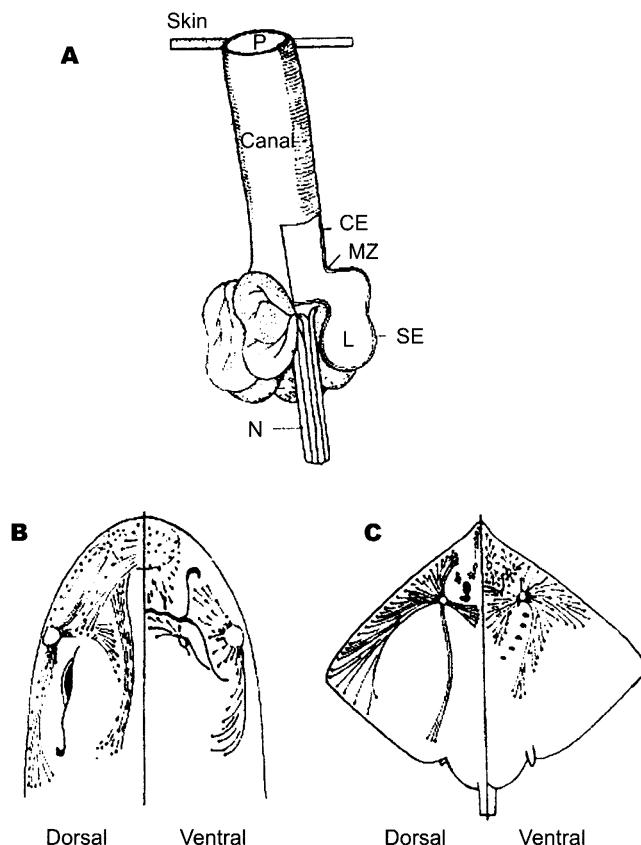
Fig. 1 The ampulla of Lorenzini system in marine elasmobranchs. A, Diagrammatic representation of the canal and ampulla that consists of several alveoli pouches. The ampulla walls are formed by the sensory epithelium (SE) that connects with the canal epithelium (CE) at the marginal zone (MZ), and is innervated by primary afferent neurons (N). The inner lumen (L) and subdermal canal are filled with a low resistive gel that form an electrical 'core conductor' and brings the lumen isopotential with the charge at the canal pore. (Modified from Waltman (1966). B, Representation of the ampullae of Lorenzini in the cat shark, *Scyliorhinus*. Ampullae are grouped into clusters in the head and have canals of different lengths that radiate in many directions. C, Representation of the horizontal distribution of ampullae of Lorenzini in the skate, *Raja*. Note that the canals project primarily within the horizontal plane of the head and pectoral fins due to the dorsoventrally flattened body. Figs. Modified from Murray (1960)

Receptor cells are flask shaped, and have a single apical kinocilium which projects directly into the lumen (Fig. 2A). Accessory (support) cells form the vast majority of the lumen surface and are bound to receptor cells by tight junctions that prevent ionic leakage between the lumen and basal portion of the epithelium. The receptor epithelium forms the thin (15 mm thick) wall of the ampulla, which is innervated in the basal region by primary afferent neurons (Fig. 2B). Tight junctions also occur around the apical surface of the cell but only a small fraction of the receptor cell surface (including the kinocilium) is exposed to the interior of the alveolus chamber. Both the canal lumen and the ampullary chambers are filled with a low resistivity,
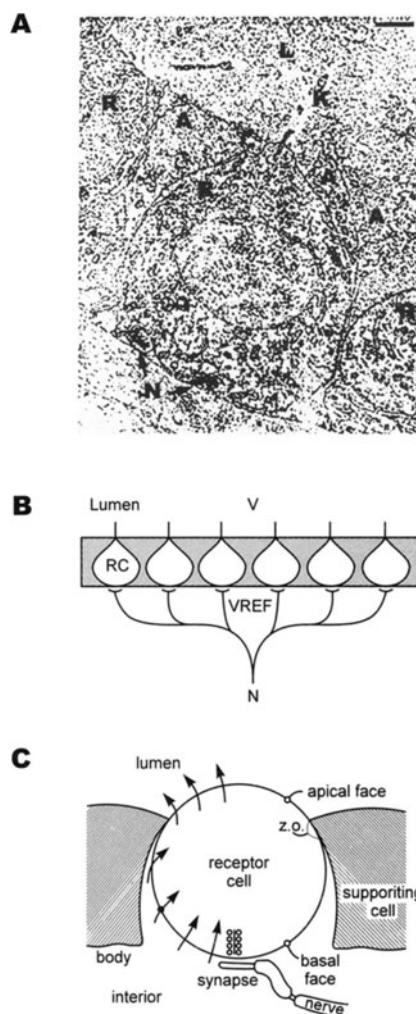
Fig. 2 Receptor cells of the ampullary electrosensory system. A, Transmission electron micrograph of the receptor cells (R) and adjacent accessory (support) cells (A) which are united by tight junctions. The kinocilium (K) projects into the ampullary lumen (L) and primary afferent neurons (N) innervate receptors at basal surface. Fig. modified from Waltman (1966). B, Diagram of the sensory epithelium. Receptor cells (RC) and adjacent accessory cells form the thin sensory epithelium layer. Tight junctions form a high electrical resistance barrier between the lumen of the ampulla and basal portion of the receptor cells. The difference between lumen voltage (V) and reference voltage (VREF) stimulates the small apical surface of the receptor cells and controls release of neurotransmitter onto primary afferent neurons (N). Fig. modified from Tricas (2001). C, Diagram of the ampullary receptor cell showing current flow during excitation. A cathodal (negative) stimulus relative to the basal region of the receptor excites the apical surface of the cell which causes an increase in outward current flow into the lumen (arrows). This in turn causes inward current at the basal region (arrows), release of chemical transmitter at the cell synapse, and excitation of the primary afferent nerve. Anodal potentials in the lumen will decrease outward current flow at the apical surface and subsequently decrease the rate of transmitter release. Modified from Bennett and Clussin (1977).

muccopolysacharrhide gel secreted by the superficial layer (Murray and Potts 1961, Waltman 1966). Each canal functions as a low-resistance conductor that provides charge at the ampullary lumen that is isopotential with that at the pore on the skin.

The tight junctions of the sensory epithelium concentrate a bias current flow that enters the receptor cells through their basal surface and exits into the lumen via the apical surface (Fig. 2C) (Obara and Bennett 1972). Receptor cells show a well-developed synapse for chemical transmission to afferent neurons at their basal surface. A single layer of transmitter vesicles covers the synaptic ribbon and releases chemical transmitter to depolarize the postsynaptic membrane of the primary afferent neuron. This regular release of neurotransmitter results in a regular pattern of neural discharge in the absence of externally applied stimuli. The receptor cells are excited by a cathodal (negative) stimulus applied at the apical surface (or skin pore) that further depolarizes the apical cell face. Regeneration of the apical membrane depolarizes the basal cell surface, which causes transmitter release into the synaptic cleft and an increased discharge rate in primary afferents. In contrast, applied anodal (positive) charges decrease the flow of bias current through the cell and results in a decrease of transmitter release onto primary afferents. There is no efferent projection from the brain to the basal region of electrosensory hair cells as occurs in the other octavolateralis sense organs (Roberts and Meredith 1989). Thus, changes in the polarity and intensity of the electric potential at the skin pore (and apical surface of the receptor cell) over time will modulate the resting discharge pattern of primary afferent neurons.

The electrosensory system of marine elasmobranchs can detect extremely weak electric fields within their environment. Murray (1962) reported neural responses of skate electrosensory primary afferents to a voltage gradient of approximately 1 μV/cm, and more recent experiments have extended this sensitivity to below 20 nV/cm (Tricas and New 1998). The neural response to a constant current field is robust, but adapts back to the resting discharge rate within a few seconds. Thus, ampullary electroreceptors show maximum responses to phasic fields delivered at frequencies from 1-10 Hz (Andrianov et al. 1984, New 1990, Montgomery 1984, Peters and Evers 1985, Tricas et al. 1995, Tricas and New 1998, Sisneros and Tricas 2000). Sensitivities of primary afferent fibers innervating ampullary electroreceptors to a uniform sinusoidal field range from 0.9 spikes/sec per μV/cm for the little skate, *Raja erinacea* (Montgomery and Bodznick 1993), 4 spikes/sec per μV/cm for the thornback guitarfish, *Platyrhinoidis triserata* (Montgomery 1984), 7.4 spikes/sec per μV/cm average for the Atlantic stingray, *Dasyatis sabina* (Sisneros and Tricas 2000a, 2002), 17.7 spikes/sec per μV/cm average for the clearnose skate, *Raja eglanteria* (Sisneros et al. 1998), and 24 spikes/sec per μV/cm average for the round stingray *Urolophus halleri* (Tricas and New 1998).

NATURAL PHYSICAL STIMULI OF THE ELECTRORECEPTOR SYSTEM

An ampullary electroreceptor responds to the difference between potentials at its apical surface (within the ampulla lumen) and basal surface (external surface of the ampulla). In some cases, ampullae are grouped into discrete clusters whereas other ampullae can be scattered across a

wide region of the head or fins. In the case of contiguous groupings of individual ampullae into distinct clusters, the receptors experience a common reference potential at their basal region (Fig.3). All sensory cells of a single ampulla experience the same apical voltage that co-varies with the potential at its skin pore. Thus, functionally the hair cells act as differential voltage detectors and stimulate primary afferent neurons as a function of the difference between potentials at the skin pore and internal potential of the animal.

The morphological arrangement of the ampullary canals permits detection of small local fields produced by biological organisms (Kalmijn 1974, Tricas 2001). Physiological processes in marine organisms result in an uneven distribution of ionic charges within the body that may produce weak standing or alternating multipole electric fields around the animal. When an adequate dipole stimulus from another organism such as a prey (e.g. Haine et al. 2001) nears a pore, the charge is conducted along the low resistance pathway of the canal interior and appears at the apical surface of the receptor cells within the single associated ampulla (Fig. 3A). This results in selective stimulation of ampulla receptors within the cluster, and depolarization of primary afferent neurons that are somatotopically mapped to the location of the pore on the skin. In this simple example, all ampullae have the same internal reference potential, are stimulated as a function of charge intensity at their respective surface pores, and have a neural response that is due primarily to the voltage drop across the skin and independent of canal length (Kalmijn 1974).

Sharks and rays can experience weak electric stimulation from fields that result from inter-actions with their environment (see Navigation section below), or when at the fringe of a large polar field. When the animal's body is within an extrinsic uniform field the low resistivity of the body admits the field to influence the internal reference potential (Kalmijn 1974). When a weak uniform field is applied along the length of the canal, the stimulus voltage in the lumen is determined by the linear separation between the ampulla and its canal pore. Thus, within a uniform field the primary afferent neurons associated with long canals receive stimulation across a greater spatial distance, are stimulated by a larger potential difference at their receptor cells, and exhibit the highest neural sensitivity (Tricas 2001) (Fig.3B).

Research on the spatial features of ampullary pores and canals on the body elucidate the importance of sensory system structure, ecology and behavior of individual species. Raschi (1986) compared anatomical characters of the ampullary system among 40 species of skate. He found that ampullary pore density was greatest in regions around the mouth probably to enhance prey capture, and that deep water species had larger (and presumably more sensitive) ampullae than did shallow water species. He also found inverse correlations between pore density and the mobility of natural prey. Tricas (2001) used a neuroecological approach to address adaptations of the electrosensory system in relation to the projection vectors of canals in the skate, *Raja laevis* and the great white shark, *Carcharodon carcharias*. This work showed that the body form and spatial arrangement of the ampullary system may set important functional constraints on the detection of natural electric stimuli. The dorsoventral compression of the batoid body limits the projection vectors of long canals to the horizontal plane. Thus the batoids can detect small dipole fields over a large surface of the body, but are mainly sensitive to the horizontal components of external fields. In contrast to the batoids, the

A

Local polar field

+ + + + − − − − 0 0 0
V1 V2 V3 V4 V5 V6 V7

Skin

VREF = Common internal

V4 V7
V1 V7
VREF

N
6
7
4
1
2

Nout ∝ VREF − Vn

B External uniform field

V1 V2 V3 V4 V5 V6 V7

+ → −

VREF ≈ V4

(Internal field)

V4 V7
V1 V7
VREF

N
6
7
4
1
2

Nout ∝ VREF − Vn

+ → −
~V2 ~V3 ~V4 ~V5 ~V6
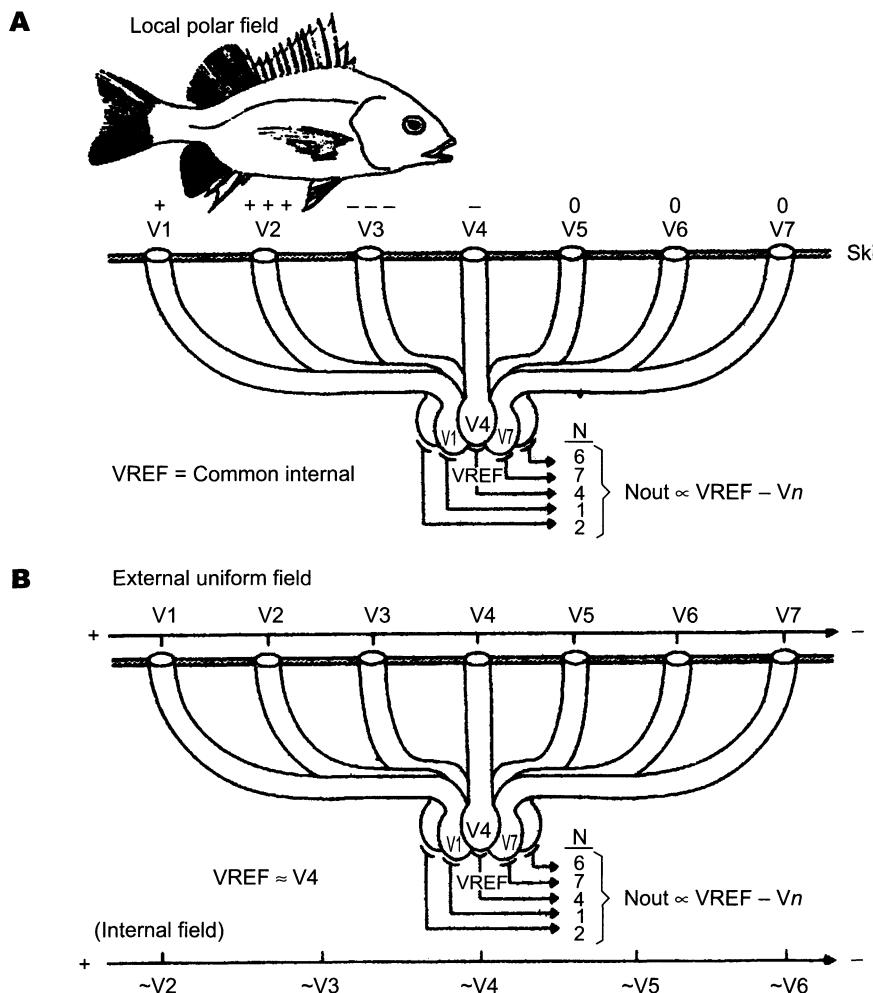
Fig. 3 Diagrammatic and simplified model for the encoding of weak polar and uniform electric fields by a subdermal cluster of ampullae of Lorenzini. A, individual ampullae are grouped in a subdermal cluster and their individual canals project beneath the skin to surface pores located over the head (and pectoral fins of batoids). A weak polar bioelectric field is produced by a prey organism (fish inset) and presents charges (+, 0, −) represented as voltage potentials at individual pores on the skin (Vn=V1 to V7). The potential at each skin pore is sampled by its conductive subdermal canal and results in an isopotential ampullary lumen (e.g. V1, V4, V7). Electroreceptors (not shown) that form the wall of a single ampulla are stimulated by the difference between the potential within the lumen and the independent internal potential located at the basal region of the receptor epithelium (VREF). Small populations of primary afferent neurons uniquely innervate each ampulla (arrows), are stimulated exclusively by the transreceptor potentials within their associated ampulla, and transmit somatotopic electric information to the brain via parallel neural channels (numbers indicated below N). The change in resting discharge rate output for each primary afferent neuron (Nout) is proportional to the difference between the common VREF and the potential at its associated surface pore. B, in the presence of a uniform field the low resistivity of the body admits a portion of the external field that also influences the common reference potential at the cluster. In this example, VREF is approximately equal to the external potential that is orthogonal to the internal field line (V4). Voltage potentials represented within the ampullary lumen (V1, V4, V7) are a function of canal length across the external field. From Tricas (2001).

ampullary canals of the white shark (and most sharks with a fusiform body) have canals that project into three-dimensional space rather than only the horizontal plane. This complex spatial arrangement provides electrosensory information about the charge distribution around the entire surface of the animal, and makes it possible for sharks to gain an image of a field in three-dimensional space. This study also showed that subgroups of canals within a single ampullary cluster have distinct projection vectors, and indicates that single clusters may serve multiple context-specific functions such as feeding and orientation behaviors.

BIOLOGICAL CONTEXTS AND BEHAVIORS

The first demonstrated biological function of the ampullary electrosense was for the detection of prey, and there is considerable theoretical work on the possible use in electric-mediated orientations. Recent experimental work has expanded functions of the electrosensory system to

Table 1 Biological functions for the electrosensory systems of sharks and rays.

Biological Function	Source
Prey detection	Kalmijn 1971; Tricas 1982; Blonder and Alevison 1988;
	Lowe et al. 1994; Haine et al. 2001; Kajiura and Holland 2002
Social communication	Bratton and Ayers 1987; Sisneros et al 1998
Detection of predators	Peters and Evers 1985; Sisneros et al. 1998
Detection of mates	Tricas et al. 1995
Geonavigation	Kalmijn 1974; Paulin 1995

include other contexts that occur during social and antipredator interactions (Table 1).

Detection of prey

The best-known function of the elasmobranch electrosense is for the detection of bioelectric fields produced by prey. Kalmijn (1971) performed a classic series of behavioral experiments in the laboratory on the catshark, *Scyliorhinus canicula*, and the Black Sea skate, *Raja clavata*, to show that elasmobranchs use the electrosense for prey localization. These fish executed well-aimed feeding responses to small flounder buried in the sand and were able to locate buried prey placed within an agar chamber that was permeable to its bioelectric field but not its odor. The ability to locate the prey was abolished when a thin plastic film that electrically insulated the field was placed over the agar chamber. These elasmobranchs also showed natural orientation responses toward buried active-dipole electrodes that produced simulated electric prey fields. Kalmijn (1982) later demonstrated in field experiments that free-ranging sharks such as the dusky smooth hound, *Mustelus canis*, and the blue shark, *Prionace glauca*, could be attracted by prey odor but would preferentially attack a weak electric dipole source (Fig. 4A). Tricas (1982) showed that in their natural habitat swell sharks, *Cephaloscyllium ventriosum*, use natural bioelectric fields to capture prey during normal nocturnal feeding (Fig. 4B). Other behavioral experiments using active dipole sources confirm the use of the electrosense for prey

detection in other species including the Atlantic stingray, *Dasyatis sabina* (Blonder and Alevizon 1988), the Pacific electric ray, *Torpedo california* (Lowe et al. 1994), the sandbar shark, *Carcharhinus plumbeus*, and the scalloped hammerhead shark, *Sphyrna leweni*, (Kajiura and Holland 2002). Collectively, these studies demonstrate that sharks and rays rely heavily upon their electrosense to locate natural prey at close range during the night or daytime, especially when prey are not in the field of view. However, because of the relatively small size of natural prey for sharks and rays and the polar nature of prey bioelectric fields, the field strength falls off quickly
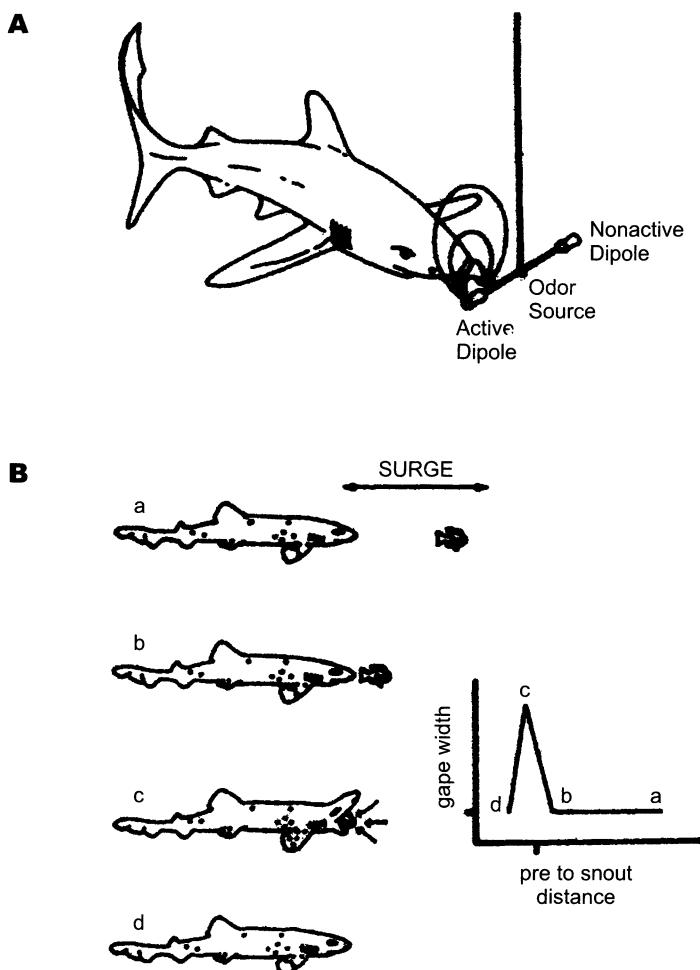
Fig. 4 Prey detection in elasmobranchs. A, Feeding response of the blue shark, *Prionace glauca*, on an active dipole source that electrically simulated prey. Blue sharks preferentially attacked the active dipole source rather than the prey odor source. (Modified from Kalmijn 1982). B, Gulp feeding response of the swell shark, *Cephaloscyllium ventriosum*, on blacksmith fish prey in the natural habitat. Sharks use an ambush predator strategy by lying on bottom at night and orient parallel to surge flow (a) and wait for blacksmith prey fish to swim near their snouts. Sharks wait until prey is within approximately 1-5 cm from the snout (b) and then "suck" into the mouth (c) and swallow fish (d). Modified from Tricas (1982).

with distance (Kalmijn 1988). Thus the effective distance of this sense in prey detection is usually limited to a distance of a few cm from the source.

Detection of conspecifics

Work on the non-electric stingrays has extended the role of elasmobranch electroreception to include social behavior. Tricas et al. (1995) showed that round stingrays, *Urolophus halleri*, use the electrosense to detect and locate conspecifics during the mating season (Fig. 5A). The main stimuli for conspecific localization are the weak bioelectric fields produced by cryptically buried females (Fig. 5B). Both males and females use their electrosense in a sex-specific context during the mating season, and orient towards buried conspecifics from distances of 0.1-1 m from the source. Male stingrays use their electrosense to detect and locate female mates, while females use the electrosense to locate and join buried conspecific females for refuge (Tricas et al. 1995, Sisneros and Tricas 2002b). Stingrays produce a standing dc bioelectric field that is partially modulated by the ventilatory movements of the spiracles, mouth and gill slits (Kalmijn 1974, Tricas et al. 1995). Both the static and modulated portions of this bioelectric field are attractive stimuli that can be used by searching stingrays to locate conspecifics buried in the substrate. The static portion of the ray's bioelectric field appears to vary at low frequency as the searching ray's electroreceptor system passes through it (sensu Kalmijn 1988). The modulated portion of the bioelectric field varies with the natural ventilatory movements of the ray ($\sim$ 0.5-2Hz) and because of the rapidly adapting nature of primary afferent discharges, may provide a significant electric stimulus especially when a receiver does not move such as occurs during inspection behavior. The peak frequency sensitivity of the electrosensory primary neurons in the round stingray matches the modulated frequency components of the bioelectric fields produced by conspecific stingrays (Fig. 5C). This match between peak frequency sensitivity of the peripheral electrosensory system and the ventilatory phasic signals produced by conspecifics indicates that the electrosense serves an important biological function in elasmobranch social behavior and that it can be used in sex-dependent contexts for conspecific localization during the mating season.

In addition to the non-electrogenic bioelectric fields, the weak electric organ discharges (EODs) of skates were proposed to serve an intraspecific communication function during social and reproductive behaviors rather than a defensive or predatory function (Mikhailenko 1971, Mortenson and Whitaker 1973, Braton and Ayers 1987). The peak frequency sensitivity of the electrosensory primary afferents in the clearnose skate, *Raja eglanteria*, is similar to the pulse rate of EODs produced by conspecific skates during social and mating behaviors (New 1994, Sisneros et al. 1998). A similar correspondence is seen between the peak frequency sensitivity of the electrosensory primary afferents in the little skate, *Raja erinacea*, and the EOD pulse rate produced by conspecifics of that species (Bratton and Ayers 1987, New 1990). Thus, the match between the electrosensory-encoding properties of the peripheral electrosensory system and the EOD pulse rate in these skates emphasizes the potential importance of the skate electrosense for electric communication during social and reproductive behaviors. However, much behavioral work remains to identify the specific social and sex-dependent contexts in which the skate EOD naturally function.

A

B

C
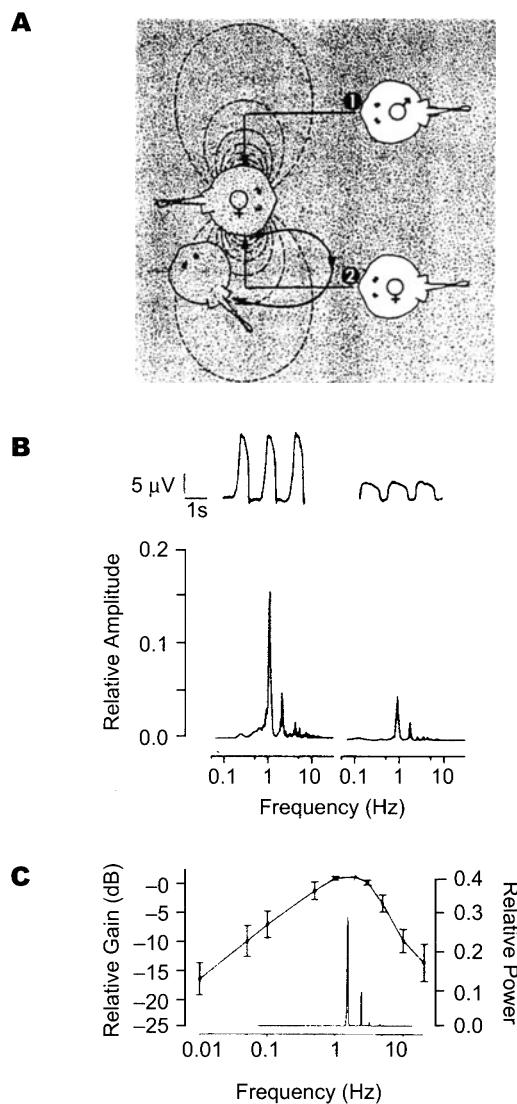
Fig. 5 Conspecific detection of mates, bioelectric stimuli, and the frequency response of the peripheral electrosense in the round stingray, *Urolophus halleri*. A, Diagrammatic representation of how male and female round stingrays use the electrosense to detect cryptically buried females during the mating season. Male rays use the electrosense to detect and locate females for mating while females use the electrosense to locate and join buried consexuals for refuge. B, Bioelectric potentials recorded from a female stingray on the ventral surface near gill slits (top, left record) and dorsal surface above the spiracle (top, right record). Recorded potentials vary part of the standing DC field and are similar for both male (not shown) and females. Fourier transforms of ventilatory waveforms (bottom) show strong frequency components near 1-2 Hz. C, Match between the peak frequency sensitivity of electrosensory primary afferent neurons and the frequency spectrum of the modulated bioelectric waveforms produced by round stingrays. The electrosensory primary afferents in *U. halleri* show greatest frequency response at approximately 1-2 Hz with a 3 dB bandwidth of about 0.5-4Hz. Data are plotted as the relative gain of mean discharge peak (± 1 SD) expressed in dB. Figs. A-C modified from Tricas et al. (1995).

Detection of predators

Another important function of the elasmobranch electrosense is for the detection of bioelectric fields produced by predators. Recent work on the clearnose skate, *Raja eglanteria*, not only demonstrates that the electrosense is functional in late-term egg-encapsulated embryos but also that their electrosense is most sensitive to the frequency spectrum that is produced by potential egg predators (Sisneros et al. 1998) such as produced by elasmobranchs, teleost fishes, marine mammals and molluscan gastropods (for review see Cox and Koob 1993). Late-term skate embryos vigorously undulate their tail in one corner of the egg case to create a continuous flow of seawater over their body for respiration (Fig. 6A) (Luer and Gilbert 1985). This tail undulating action draws fresh seawater through the egg case and creates a hydrodynamic streaming of seawater from the exit pore that can provide olfactory, electrosensory and mechanosensory cues to potential predators. The peak sensitivity of electrosensory primary afferent neurons in skate embryos is at the same frequency as the phasic ventilatory electric signals (0.5-2 Hz) produced by large fish predators, interrupts the respiratory movements of embryonic skates, and elicits an antipredator freeze behavior (Fig. 6B,C) (Sisneros et al. 1998). The freeze response exhibited by skate embryos stops the ventilatory streaming of seawater from the egg case and decreases the likelihood of olfactory, electrosensory, and mechanosensory detection by predators. Phasic electric stimuli of 0.1 to 1 Hz are also known to interrupt the ventilatory activity of newly post-hatched catsharks, *Scyliorhinus canicula* (Peters and Evers 1985), and this electrosensory-mediated behavior may represent an adaptive response during early life history to avoid detection by predators and enhance survival. Of potential significance is that a polar bioelectric field produced by a large predator would be strong compared to that of a natural prey item, but the effective distance for electrosensory detection of potential predators remains to be experimentally determined.

Navigation

The electrosense of elasmobranchs is known to mediate orientation to local inanimate electric fields and in theory is sensitive enough to function in geomagnetic navigation. Pals et al. (1982a) showed in behavioral experiments that the catshark, *Scyliohinus canicula*, could use dc electric fields to orient within a captive environment without light. Kalmijn (1982) also demonstrated that round stingrays, *Urolophus halleri*, orient within a uniform electric dc field, discriminate the direction of the dc field based on its polarity, and detect voltage gradients as low as 5 nV/cm. The electric field gradients used in the above experiments were of magnitudes similar to those produced by ocean currents (500 nV/cm, Kalmijn 1984) and tidal currents (8 μV/m, Pals et al. 1982b).

According to Kalmijn (1974, 1981, 1984), elasmobranchs may use the electrosense for two modes of electronavigation. In the passive mode, elasmobranchs detect the voltage gradient produced in their external environment such as occurs during flow of ocean water through the earth's geomagnetic field (Kalmijn 1988). In the active mode, elasmobranchs detect voltage gradients that are induced by the animal's locomotor movements through the earth's magnetic
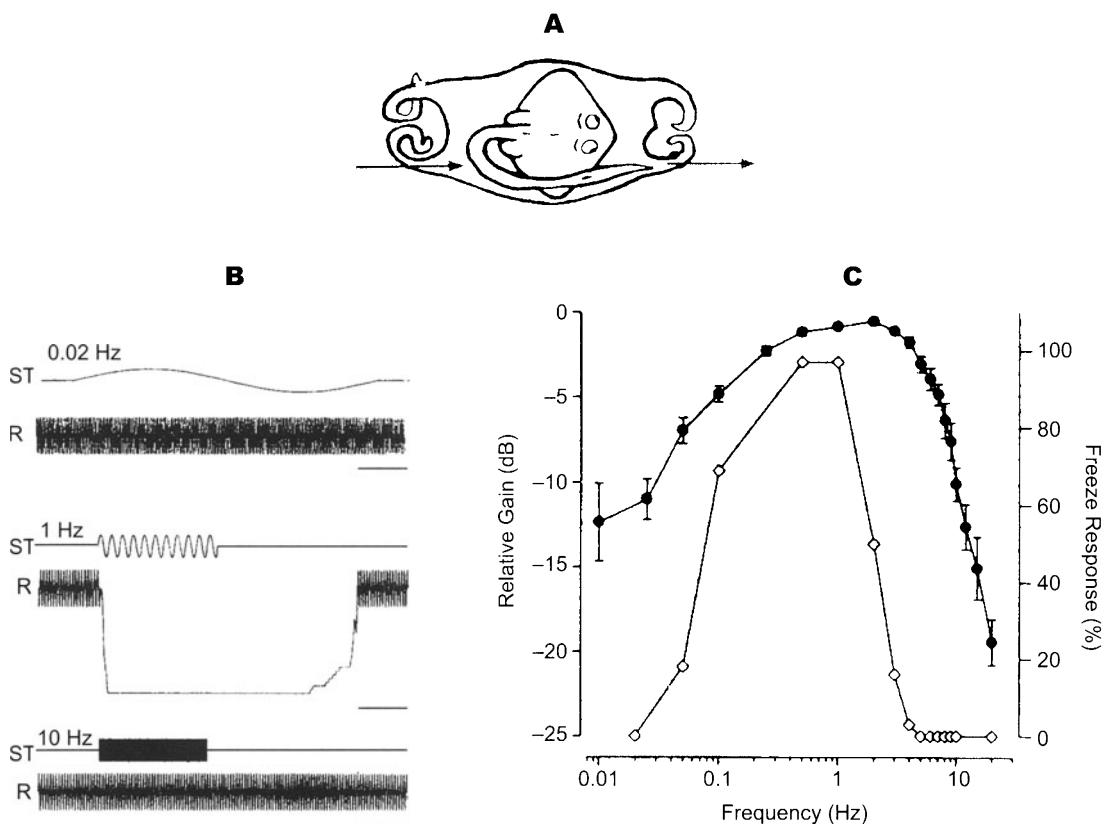
Fig. 6   Behavioral response of clearnose skate embryos, *Raja eglanteria*, to weak electric stimuli. A, Ventilation behavior of skate embryos. Diagram depicts a late-term embryonic skate that drives seawater through the egg case by undulating its tail near a ventilation pore in the horn. The tail beating action of the skate draws fresh seawater through pores on the opposite end of the case and creates a localized vortex near the exit pore by the tail. Arrow indicates flow of seawater. Modified from Sisneros et al. (1998). B, Behavioral responses of embryonic skates to sinusoidal uniform electric fields at stimulus (ST) frequencies of 0.02 Hz, 1Hz and 10 Hz. Stimuli were applied at an intensity of 0.56 nV/cm across the longitudinal axis of the skate. The response (R) is expressed as a change in the peak-to-peak (PTP) tail displacement within the egg case. Prestimulus tail displacement for each record was 10 mm PTP. At 1 Hz, note the large tail displacement that occurs during coiling of the tail around the body after the onset of the electrical ST and a period of no tail movement during and after stimulation. Time bars = 5 seconds. C, Freeze response of skate embryos to weak electric stimuli. Behavioral responses (open diamonds) are shown as a percentage of total ST presentation to 0.02-20 Hz. Note that the peak frequency sensitivity of electrosensory primary afferent neurons (solid dots) for skate embryos is at 1-2 Hz and is aligned with the freeze response peak of 0.5-1Hz. Figs. A-C were modified from Sisneros et al. (1998).

field (Fig. 7). Another active mode model by Paulin (1995) proposes that electrosensory and vestibular canal information during head rotation are integrated and should provide unambiguous directional cues during swimming. However, direct experimental tests of these active mode models await to be performed.
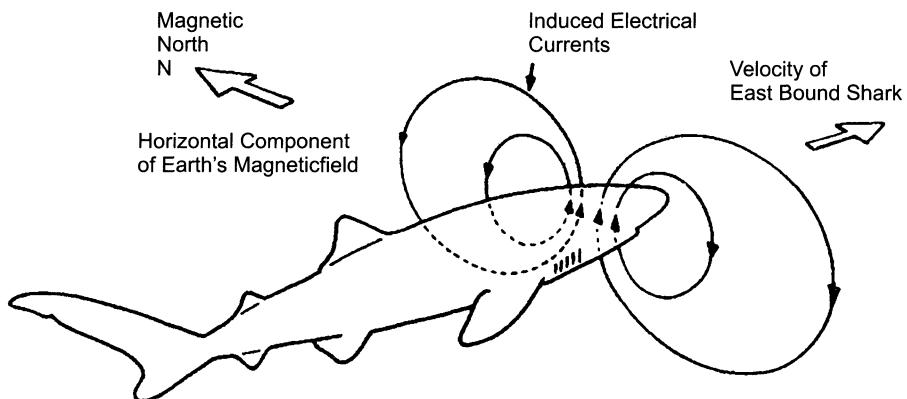
Fig. 7 Active mode induction of weak electric fields at the head as a shark swims through the horizontal component of the earth's magnetic field. This can provide a physical basis for an electromagnetic compass sense. From Kalmijn (1974).

Experimental evidence is consistent with the ability of elasmobranchs to use magnetic field information for electric orientation and navigation. Kalmijn (1982) showed that in the absence of an imposed electric field round stingrays, *U. halleri*, could be conditioned by food reward to locate and enter an enclosure in the magnetic east and to avoid an enclosure in the magnetic west. In addition, stingrays could discriminate the direction and polarity of the magnetic field. More recently Klimley (1993) showed that scalloped hammerhead sharks, *Sphyrna leweni*, swimming in the pelagic realm followed fixed, repetitive homing routes that correlated with the ambient pattern of geomagnetic anomalies associated with the ocean floor and he proposed that they may navigate by the use of geomagnetic fields. However, further experiments are needed to determine whether this orientation is mediated by direct magnetoreception or induced geomagnetic electroreception.

ONTOGENETIC AND SEASONAL CYCLES

Sharks and rays are large, long-lived fishes that can inhabit different ecological habitats during their life history. In addition, seasonal changes in behaviors such as migration or reproductive activity may result in seasonal variation in biological functions for the electrosense. Recent research has begun to address questions on temporal and spatial changes in electrosensory function.

Age-dependent effects on electrosensory responses

Physiological studies indicate that the ampullary electroreceptors in adult elasmobranchs are broadly tuned to low-frequency electric stimuli and respond maximally to sinusoidal stimuli from approximately 0.1 to 15 Hz (Andrianov et al. 1984, Montgomery 1984, Peters and Evers 1985, Tricas and New 1998). However, recent neurophysiological work on the clearnose skate, *Raja eglanteria*, and the Atlantic stingray, *Dasyatis sabina*, indicates that the discharge and

bandpass filtering properties of the peripheral electrosense change during ontogeny (Sisneros et al. 1998, Sisneros and Tricas 2002a). The resting discharge rate and regularity of the electrosensory primary afferents in these batoids increase with age and may serve to enhance the temporal resolution for encoding of low-frequency electrosensory stimuli (Sisneros and Tricas 2002a). Furthermore, during development the tuning properties of the peripheral electrosensory system shift to higher frequencies and sharpen. The –3 dB bandwidth of the peripheral electrosense in the Atlantic stingray is ~2Hz higher in adults (2.7-10.1 Hz) than in neonates (1.1-8.5 Hz). In addition, the –10dB bandwidth in Atlantic stingrays narrows from 1.1-28.7 Hz in neonates to 0.5-18.5 Hz in adults (a decrease in bandwidth by ~10 Hz). (The –3dB and –10 dB bandwidths are measures that describe the range of frequencies over which the sensitivity of the electrosensory system falls within the prescribed limits. In this case, the band-widths of the peripheral electrosensory system are described as the range between frequencies at which the response is –3dB and –10dB compared with the peak response at midband.) Concurrent with the ontogenetic shift in –3dB bandwidth is an increase in best frequency (i.e. the frequency that evokes the maximum response from the electrosensory primary afferents) from 2-4 Hz in neonates to 3-5 Hz in juveniles, and 6-8 Hz in adults (Sisneros and Tricas 2002a). Similar ontogenetic shifts in –3 dB bandwidth and best frequency also occur in the clearnose skates, *Raja eglanteria* (Sisneros et al. 1998).

In addition to changes in the frequency response properties, the neural sensitivity (gain) of electrosensory primary afferents also increases with size during ontogenty in *R. eglanteria* and *D. sabina* (Sisneros et al. 1998, Sisneros and Tricas 2002a). Sensitivity in the clearnose skate is approximately five times greater in juveniles (mean total length = 17.4 cm) and eight times greater in adults (mean total length=52.3 cm) than in embryos (mean total length = 11.9 cm) (Sisneros et al. 1998). Sensitivity at best frequency in the Atlantic stingray is approximately three times greater in juveniles (mean disk width = 15.1 cm) and four times greater in adults (mean disk width = 25.0 cm) compared to neonates (mean disk width = 11.6 cm) (Sisneros and Tricas 2002a). As young batoids grow in size, an increase in sensitivity is expected due to the concurrent increase in canal length. The sensitivity of electrosensory primary afferents to uniform electric fields is positively correlated with canal length (Fig. 8) (Sisneros and Tricas 2000a), and therefore accounts for a large part of the observed increase in neural sensitivity through growth.

The adaptive importance of the ontogenetic changes in the response properties of the peripheral electrosense may be related to complementary functions during development to avoid predation and maximize prey detection. As discussed above, the peak frequency response of embryonic and neonate batoids is within the peak frequency band of phasic potentials produced by natural fish predators, corresponds to the same frequency stimuli that interrupt respiratory movements (Sisneros and Tricas unpublished data), and elicits an antipredator freeze response in embryonic skates (Sisneros et al. 1998). Such ontogenetic shifts in the frequency tuning of batoids may also affect a shift in foraging behavior and the diet of inverte-brate prey. Infaunal prey such as polychaete worms and bivalves emit predominately unmodulated DC fields (Kalmijn 1974) as opposed to the modulated bioelectric fields
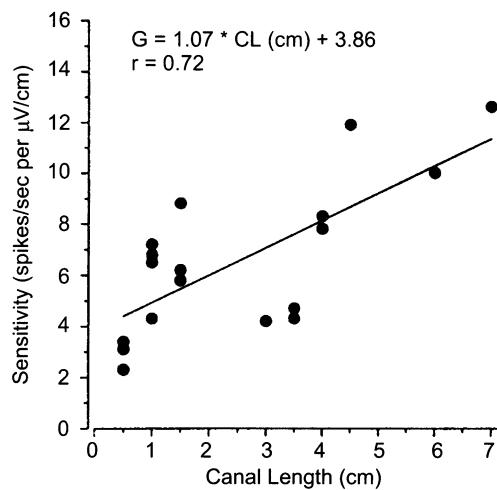
Fig. 8 Relationship between the sensitivity (gain) of electrosensory primary afferent neurons and the canal length of ampullary electroreceptive organs in the Atlantic stingray, *Dasyatis sabina*. Data are from male stingrays collected during the nonreproductive summer months. Note that the sensitivity (G) of the primary afferent neurons increases with ampullary canal length (CL). Neural gain is expressed as spikes per second (s/s) per unit of field intensity. Data replotted from Sisneros and Tricas (2000).

produced by small crustacean prey such as *Daphnia* and amphipods that generate rhythmic AC potentials at 8-10 Hz (Wilkens et al. 1997, Wilkens this volume). The AC signals from the latter prey are close to the peak frequency sensitivity of the electrosensory primary afferents in juveniles and adult batoids (Sisneros et al. 1998, Sisneros and Tricas 2002a). Weak AC signals may be important for the detection of crustacean prey, especially in cases where a batoid is at rest near a prey source and the detection of DC fields becomes difficult due to the rapidly adapting nature of electroreceptors to a constant current field. Small crustacean prey such amphipods, mysids and isopods form a major component of the diet in the Atlantic stingray, especially during the summer months when stingrays forage in the sea grass beds found seasonally in Florida lagoons. Thus, the ontogenetic changes in the response properties of the elasmobranch electrosense may represent sensory adaptations to enhance the avoidance of large predators as young, and as adults increase the probability of detecting the higher frequency information associated with small infaunal prey.

Seasonal hormonal cycles and electrosensory responses

Gonadal steroids are known to have important effects on the brain and behavior (Kelly 1982, Arnold and Gorski 1984) but very little is known about how the electrosense in sharks may be influenced by seasonal changes in hormone levels. Recent work on the reproductive biology and steroid cycles in the Atlantic stingray, *D. sabina*, provides the first look at how the electrosensory system function can change during the mating cycle in elasmobranchs. Females of this species undergo a 5-6 months period of egg development that begins in the fall and ends
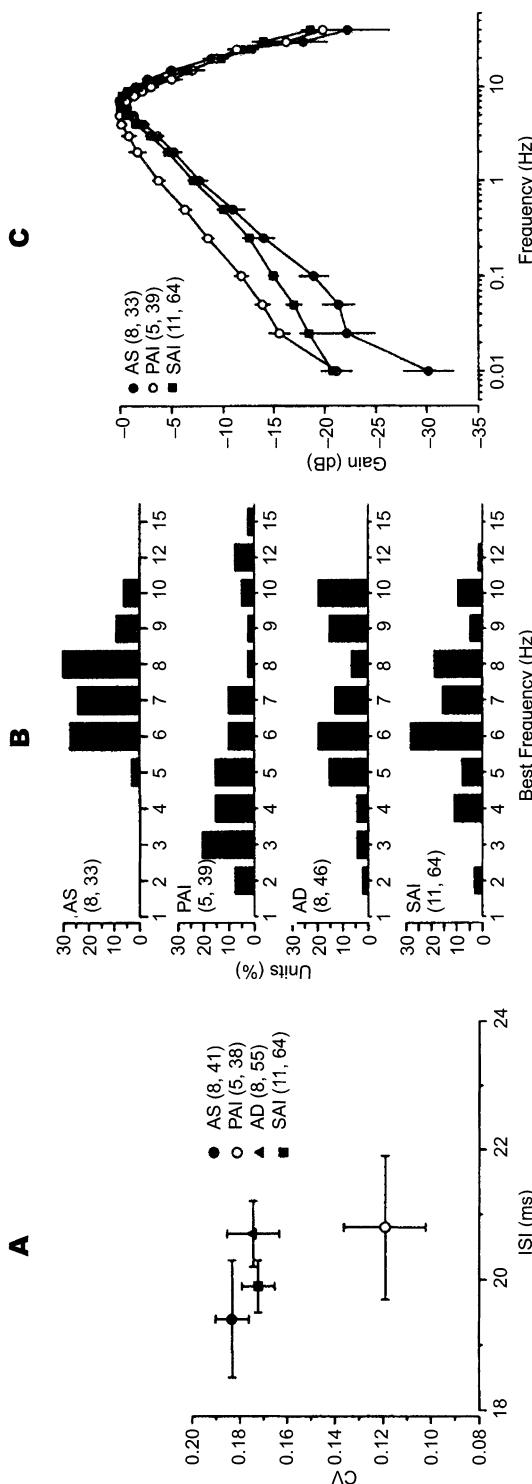
Fig. 9 Seasonal changes in the response dynamics of ampullary electrosensory primary afferent neurons in male Atlantic stingrays, *Dasyatis sabina*. A, Relationship between resting discharge variability and mean interspike interval for electrosensory primary afferent neurons in wild caught male stingrays. Rays were collected during the four phases within the annual androgen production cycle: (1) androgen suppression (AS), which occurs between reproductive seasons (April-July) during which the serum androgen levels are low and testes are inactive, (2) primary androgen increase (PIA), which occurs at the onset of the mating season and spermatocyte development (August-October), (3) androgen decrease (AD), which occurs after maximum testis growth and spermatocyte development (November-December), and (4) secondary androgen increase (SAI), which occurs at the end of the mating season and peak period of sperm maturation (January-March). Discharge variability is expressed as coefficient of variation (CV), a dimensionless ratio of standard deviation to mean interspike interval (ISA). Note the decrease in CV for PAI indicates an increase in discharge regularity during the onset of the reproductive season. The number of stingrays and electrosensory primary afferent neurons tested are indicated in parenthesis. All data plotted as mean ± standard error. B, Best frequency histogram for electrosensory primary afferent neurons recorded from male stingrays collected during annual periods of AS, PAI, AD and SAI. Number of rays and electrosensory primary afferent neurons tested are indicated in parenthesis. Note the decrease in best frequency for males collected during PAI at the onset of the reproductive season, and increased percentage of units with low best frequency. C, Bode plot for the frequency response of electrosensory primary afferent neurons recorded from male stingrays collected during annual periods of AS, PAI, AD, and SAI. Only data for males collected during AS and SAI are plotted for comparison with males collected during PAI. The number of rays and electrosensory primary afferent neurons tested are indicated in parenthesis. Peak sensitivity for males during PAI is 4-5 Hz and 7-8 Hz during AS and SAI. Data were calculated from period histogram analysis and are plotted as the mean discharge peak. In order to control for absolute sensitivity of different units, data were normalized to a relative value of 0 dB assigned to the peak response for each unit and then expressed in relative dB. All data plotted as mean ±1 standard error. Note some standard error bars are obscured by symbols. Figs. A-C modified from Sisneros and Tricas (2000).
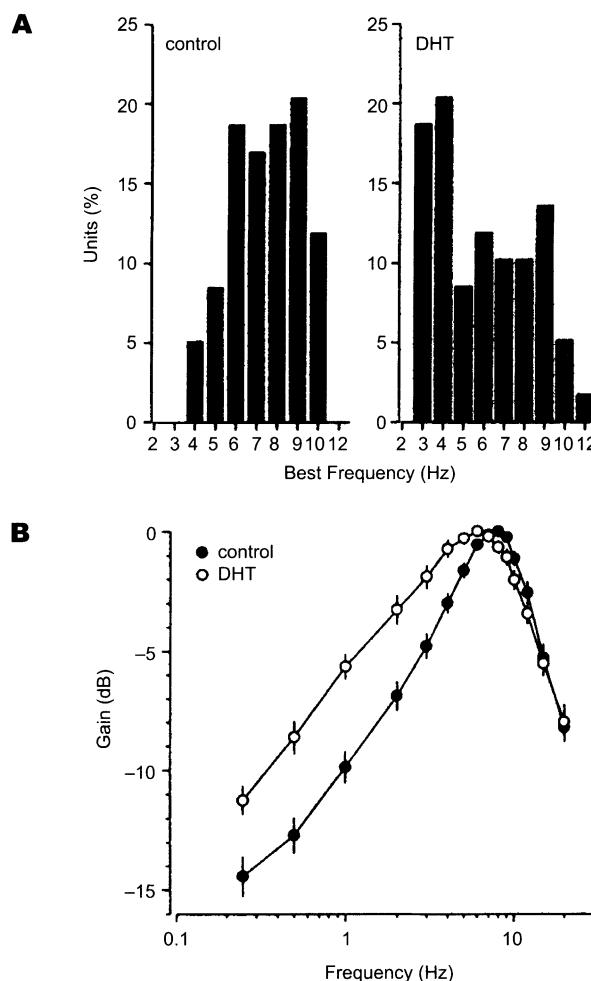
Fig. 10 Frequency response dynamics of electrosensory primary afferent neurons in control and dihydrotestosterone-treated male Atlantic stingrays, *Dasyatis sabina*. A, Best frequency (BF) histogram for electrosensory primary afferent neurons in control and dihydrotestosterone (DHT) treated male stingrays. Number of rays and electrosensory primary afferent neurons tested are indicated in parenthesis. Note that there is an induced downward shift in BFs of electrosensory primary afferents in DHT treated rays. B, Bode plot for the frequency response of electrosensory primary afferent neurons recorded from control and DHT-treated male stingrays. Peak frequency sensitivity of the electrosensory afferents is 7-8 Hz for control treated fish and 5-6 Hz for DHT treated fish. Number of rays and electrosensory primary afferent neurons tested are indicated in parenthesis. Data were calculated from the period histogram analysis and are plotted as the mean discharge peak. In order to control for absolute sensitivity of different units, data were normalized to a relative value of 0 dB assigned to the peak response for each unit and then expressed in relative dB. All data are ploted as mean ±1 standard error. Note some standard error bars are obscured by symbols. Figs. A & B modified from Sisneros and Tricas (2000).

with synchronous ovulation by all females in the spring (Maruska et al 1996). Male and female rays have a protracted mating season that completely overlaps the period of egg development (Kajiura et al. 2000). The onset of reproductive activity and egg development is accompanied by an elevation of androgen hormones in males and continues throughout the reproductive season (Tricas et al. 2000). At the beginning of the mating season, electrosensory primary affer-ent neurons in male rays exhibit an increase in resting discharge regularity, a downshift in best frequency and bandpass, and a greater sensitivity to low-frequency electric stimuli (0.01-4Hz) (Fig. 9) (Sisneros and Tricas 2000), which is similar to signals produced by conspecific mates (Tricas et al. 1995). The initiation of mating behavior and changes in the response properties of the peripheral electrosensory system in male stingrays coincide with the onset of spermatocyte production and the annual peak in androgen steroid levels for the population (Tricas et al. 2000, Sisneros and Tricas 2000). Experimental implants of dihydrotestosterone in non-repro-ductive male stingrays induced similar response shifts in electrosensory primary afferents that included a lowered best frequency and bandpass, and an increased sensitivity ($1.5\times$ increase) to low frequency stimuli from 0.5 to 2 Hz (Fig. 10). This androgen-induced plasticity of the male's electrosense may function to seasonally increase the probability of conspecific mate detection and localization during the mating season, and ultimately increase individual male fitness.

SUMMARY AND CONCLUSIONS

The electrosensory system of sharks and rays serves a wide range of natural functions including the location of prey, avoidance of predators, the detection of mates, social communication, and possibly geonavigation. These biological roles result from a highly specialized receptor system and the spatial arrangement of pores and canals on the body. The response properties and function of the electrosense may change with age and are associated with ontogenetic shifts in habitat use, foraging behavior and diet. The response properties of electrosensory neurons may change seasonally in reponse to gonadal steroid levels in the body that relate to reproductive activity and other seasonal behaviors. Future work should continue to investigate the biological contexts in which the ampullary electrosense is used, and to test the selective forces that may have shaped the evolution of this remarkable sensory system.

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Electroreception: Strategies for Separation of Signals from Noise

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ABSTRACT

The electric sense is ancestral to fishes and is present in most non-teleosts as well as certain teleost species. The electrosensory world of fishes is rich with electric fields from a multitude of sources including the earth's magnetic field and the bodies of all aquatic organisms including the electrosensing fish, itself. The fish's exquisite sensitivity to these fields allows it to orient, navigate, communicate and even detect and localize other fish, both prey and conspecifics. Electroreceptors are of two basic morphological types, ampullary and tuberous. Ampullary receptors, the ancestral type, are sensitive to extremely weak, low-frequency electric signals. Tuberous receptors represent a specialization of the weakly electric teleost fishes and are most sensitive to higher frequencies near to the frequency of the fish's own electric organ discharge (EOD).

Key words: Ampullary Organ, Tuberous Organ, Electric Organ Discharge, Gymnotiform, Mormyrid, Catfish

INTRODUCTION

Electric fields are ubiquitous features of aquatic environments, and ancestral vertebrates evolved specialized sensory organs and associated brain structures that detect these fields, presumably because the fields could be exploited to provide useful information. While not all natural electric fields may be meaningful, the electric sense is exquisitely sensitive and has developed a number of adaptations to successfully extract a 'signal' from the background (Montgomery and Bodznick 1999). In addition, several groups of fishes have separately evolved a means of generating their own electric discharge by which they can both communicate and probe the environment for electrical clues to their surroundings. The electrosensory world is rich and varied, but has only come to the attention of the non-electrosensory human biologist in the last half century. From the first experiments of Lissmann and Machin that demonstrated the ability of *Gymnarchus* to generate a weakly electric field and to use it for electrolocation

(Lissmann 1951; Lissmann and Machin 1958) a fertile literature has blossomed ranging from natural history to sub-cellular neurophysiology. A framework for further exploration in electrosensory ecology is set out below and could be used in conjunction with several recent reviews and conference proceedings (see: Bullock and Heiligenberg 1986; Kramer 1990; Moller 1995; Turner et al. 1999).

WHAT ARE THE SOURCES AND CHARACTERISTICS OF NATURAL ELECTRIC FIELDS?

A brief overview of the major sources and defining characteristics of natural electric fields is provided below (see Kalmijn 1974 for more detail). The field strengths and spectral characteristics of natural sources are often in strong overlap with the electroreceptive sensitivities of fishes that are described in later sections.

Atmospheric Sources

Lightning strikes somewhere on earth about 100 times each second, emitting broad-band pulses of electromagnetic energy that propagate within the atmosphere as waves nearly parallel to the earth's surface. These broadband pulses of energy travel around the globe within a natural waveguide formed by the earth's surface and its ionosphere (Holzworth et al. 1984). Transmission of frequencies below the waveguide resonance frequency of about 2-3 kHz is favored and attenuation through absorption by the ionosphere is on the order of only 1-2 dB Mm^{-1}, increasing with frequency. Certain frequencies, known as 'Schumann resonances' and including approximately 7.8, 14.1 and 20.3 Hz, propagate more strongly and are prominent spectral features. The principal electric and magnetic fields are vertical and horizontal, respectively, and uniform. Inhomogeneities in the fields result from nonuniform surface conditions of the earth and its ionosphere. For example, the thickness of the atmospheric waveguide varies daily between about 45 (day) and 90 km (night) leading to an increased daytime attenuation. Large nighttime disturbances (attenuation increases of 4-8 dB within several hours) following magnetic storms may last for several days.

A portion of this energy is refracted downward into natural waters as well as along the bottom and then upwards through the water column, giving a horizontal electric field. The electric field is greatly attenuated by, and in direct proportion to, the water's natural conductivity. Lower frequencies (say, below 100 Hz) are least attenuated, resulting in their penetration to greater depth and reappearance near the bottom in near-shore oceanic waters (100s of meters in depth; Soderberg 1969). Working in a stream in Gabon, West Africa, Carl Hopkins (1973) recorded broadband bursts of electrical energy from distant lightning strikes that exceeded an estimated electrosensory threshold several times each second (Fig. 1). The bursts' spectral and temporal characteristics strongly overlapped those of the electric organ discharges (EODs) of resident weakly electric fish and thus may interfere with electroreception.
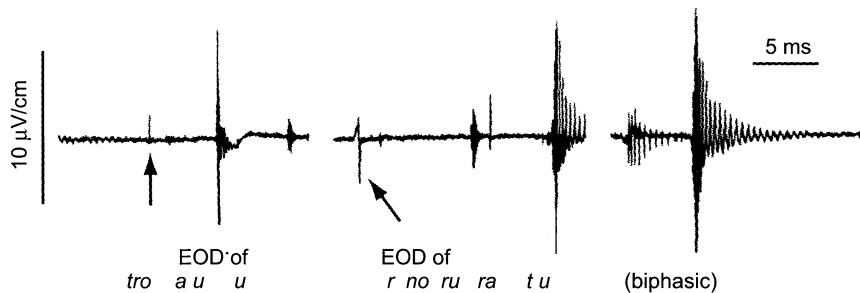
Fig. 1 Lightning from near and far is a constant source of electrical noise in the aquatic environment. These evening recordings of distant lightning were made by Carl Hopkins from a stream in Gabon, west Africa at a time when there was no atmospheric lightning in the immediate vicinity. The spectral properties of the lightning greatly overlap, and the magnitude exceeds the EODs of two species of mormyrid fishes that were also recorded (arrows). Modified from Hopkins (1986).

Oxidation/reduction Potential from Sediments

Shallow waters may have high concentrations of dissolved oxygen in the water column as a result of high photosynthetic production of O_2 and wind- and wave-induced mixing. Oxidation-reduction potentials of the oxygenated water column may be as high as +500 mV in freshwater (Koch-Rose et al. 1994) and +250 mV in estuarine waters (Reimers et al. 2001). Diffusion of O_2 from the overlying water to the bottom sediment creates an oxygenated microzone within the sediment whose depth will depend upon biological activity, temperature and O_2 demand. The thin oxidized zone is underlain by a reduced layer where a negative 'redox' potential of several hundred millivolts can be found. Thus a gradient of 6-700 mV can occur across a few centimeters or less of the water/sediment interface. The stability of the gradient will depend upon a wide range of variables such as insulation, temperature, productivity, wind and current stress to both the water column and sediments.

Low Frequency and d.c. Electric Fields Arising within the Water

Natural, very low-frequency electric fields with magnitudes on the order of tenths of μV cm^{-1} arise within natural waters (Bogorov et al. 1969). These fields are spatially complex and variable, ranging from millimeters to kilometers in scale, and arise from various physical, chemical and biological processes. So-called 'earth currents' arise from daily variations in the earth's geomagnetic field and other geomagnetic phenomena. Earth currents are generally horizontal and distributed in proportion to conductivity and are on the order of 0.01 to 0.1 μV cm^{-1} in the tropical oceans, higher in polar regions and in freshwater. Local anomalies in electrical potential as large as 5 mV have been attributed to localized differences in salinity. Large blooms of phyto- and bacterio-plankton are known to underlie voltage gradients of at least 0.4 μV cm^{-1}. These gradients are likely due to the leakage of ions during normal metabolic processes and thus probably reflect the recent localized primary production. 'Streaming currents' arise when two ionically dissimilar fluids slide across one another, such as a stream over its streambed or a fish through the water.

'Motional' electric fields with induced voltage gradients on the order of 0.05 to $0.5\mu Vcm^{-1}$ (von Arx 1962) arise from water flowing through the earth's magnetic field. Bulk flows of water moving horizontally through the earth's magnetic field will induce an electric current that is perpendicular to both the magnetic field and the direction of water flow. This induced electrical current gives rise to an electric field in the opposite direction. The strength and direction of the induced field can be found as the cross product of the vectors describing the velocity of water flow (**v**) and the perpendicular component of the magnetic field $(\mathbf{B}\perp)$[1]:

$$\mathbf{E}_{induced} = \mathbf{v} \times \mathbf{B}\perp \tag{1}$$

The earth's magnetic field is a complex vector field being almost entirely vertical at the poles and primarily horizontal at the equator. The component that is vertically perpendicular to the stream flow drives an electric current horizontally across the flow. This cross-stream electric current is returned through a separate region of different velocity, generally the slower waters at depth and the underlying sediments. This return current sets up a resistive field $\mathbf{E}_{resistive} = -\rho\mathbf{J}$ that depends upon the current density (**J**) and resistivity (ρ) of the return path.

The real ocean is somewhat more complex, however (Sanford and Flick 1975). Where waters are deep and resistivity is low, the resistive current may be roughly equal to the induced component. In more confined waters, where the water flow is effectively insulated from the stationary environment, the resistive current is minimal, only a fraction of the induced current. Motional fields become even more complex due to inhomogeneities in water flow and in the underlying resistive pathways. As well, resistive currents in one area can result from induced motional currents in another area.

Fields of Biological Origin

Complex spatial patterns of stationary electric fields are generated from most organisms as ions leak through semi-permeable and/or damaged membranes. These 'direct current' (d.c.) fields may be deeply modulated by ventilatory and other movements. As the mouth, spiracles, and/or gill slits are opened and closed, the standing d.c. potential between the internal and external environments is variously shunted. Electric fields of damaged specimens are typically one to several orders of magnitude stronger. In fish, the strongest standing ionic potentials originate from the gills, mouth, and anus with weaker potentials arising from the skin (Fig. 2D). Fields recorded just external to the bodies of elasmobranchs are typically in the ± 1-10 µV range (to perhaps 50 µV) whereas the electric fields of teleosts are regularly an order of magnitude stronger, presumably because of their different strategies for maintaining an electrolyte balance (Kalmijn 1974; Peters et al. 1974). Freshwater and marine teleosts produce similar strength fields, indicating a difference in current density of one to two orders of magnitude. Similarly modulated bio-electric fields have been recorded within a few millimeters of other potential prey, both vertebrate and invertebrate, including frog tadpoles (to several hundred µV d.c. modulated about 50%), dragonfly nymphs (150 µV d.c. with 60-70% modulation) and chironomid larvae (4 µV d.c. measured end-to-end) (Peters and Bretschneider 1972).

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[1]Vector quantities are given in **bold** and scalar (magnitude) quantities are *italicized.*

ELECTROSTATICS

Most electric fields that are important to aquatic organisms arise from sources that are of much smaller dimension than their emitted wavelength. For this reason, the fields are electrostatic in nature; they are radial and nonpropagating. Although their sources may be spatially and temporally complex, any variation in the field with distance depends on the specifics of the source- there is no absorption or refraction, and any magnetic component can be ignored. A brief review of electrostatics is set out below (for further review see, for example, Paul and Sasser 1987).

Our world is held together in large part by the strong attractive forces between positively charged protons and negatively charged electrons. These forces are in approximate worldwide balance giving an overall electrical neutrality. Locally, however, imbalances may exist when one area has more electrons or protons than another area resulting in an area of net charge either positive or negative, respectively. An unbalanced charge will exert a force on any other charge, repelling like charges and attracting opposite charges. The *magnitude* of this force (**F**), typically expressed in units of Newtons, is described by Coulomb's law:

$$|\mathbf{F}| = \frac{1}{4\pi\varepsilon_0} \frac{q_1 q_2}{r^2}. \tag{2}$$

Where q_1 and q_2 are the magnitude of the two charges (in Coulombs) that are separated by a distance, r, meters[2]. This electrical force is a vector quantity acting along the line connecting the two charges, either drawing them together or forcing them apart. The electrical force produced by multiple charges separated in space acting on one charge is the vector sum of the individual forces from each charge.

It can be convenient to think of a one Coulomb 'test charge' that allows measurement of the electrical force exerted by a given real charge. Using Coulomb's Law and by placing the unit test charge at any place in space, we can compute the force acting on the test charge. By moving the test charge to each place in space we can map the distribution of this electrical field (**E**). The electrical field has units of Newton/Coulomb or, equivalently, volts/meter[3] and the force exerted on an arbitrary charge, Q, is simply $\mathbf{F} = Q\mathbf{E}$. The electrical field can be thought of as the flux of electrical force outward (or inward) from a charge and visualized as lines of flux (Fig. 2A). Since field lines reflect the force of one charge upon another charge, they start on positive charges and stop on negative charges. No lines can start at a distance from the charge and thus the density of lines necessarily diminishes with distance from the source. The electric field intensity represented by the density of field lines thus diminishes with distance as the surface area of a sphere $(4\pi r^2)$ surrounding the field's origin.

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[2]The constant, ε_0 is called the "permittivity of free space" (8.85×10^{-12} Coulombs^2/Newton meter^2 in a vacuum).

[3]Voltage is a measure of work per unit charge and has units of (Newton meter)/Coulomb

A

monopole

B

dipole

C

EOD field lines

D

iso-potential lines
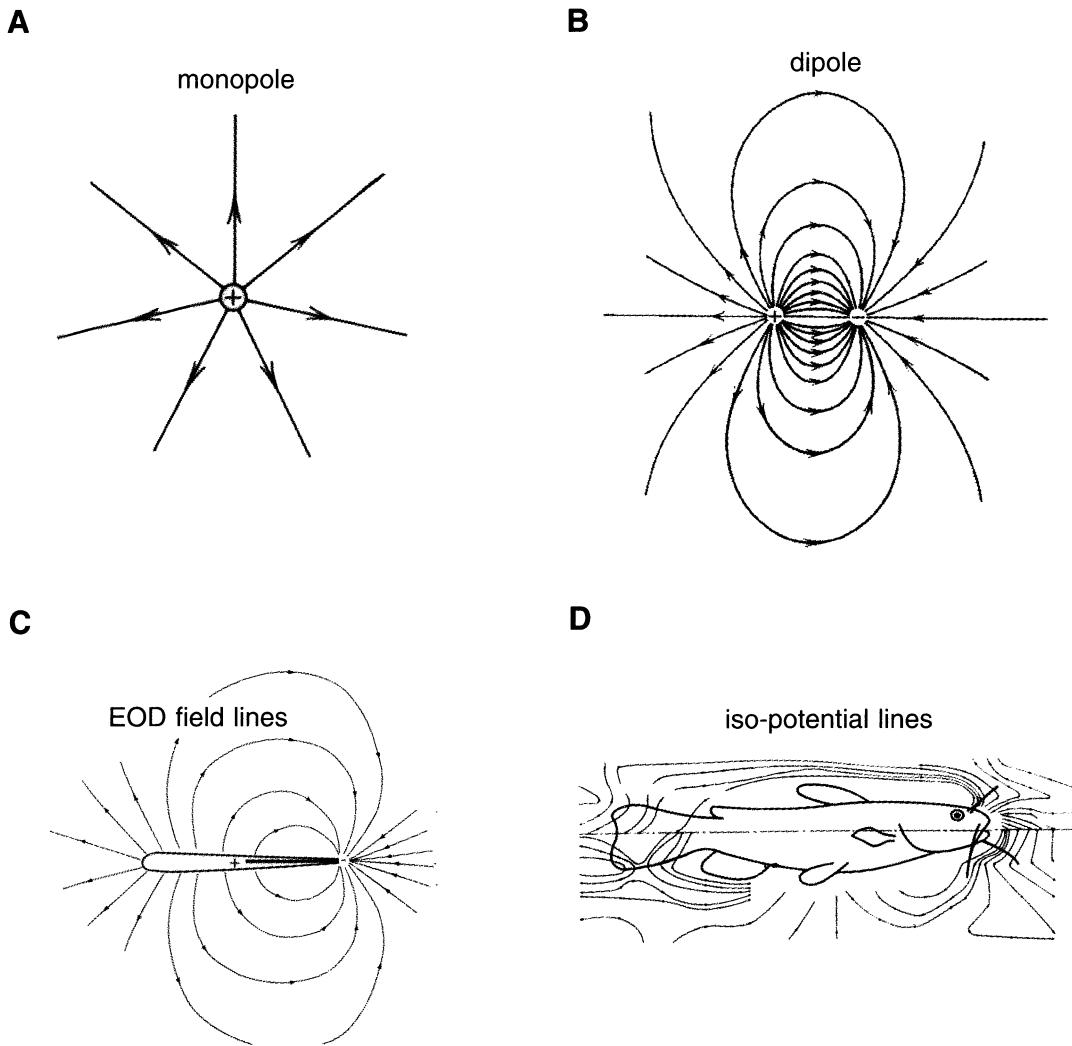
Fig. 2 Lines of electrical force start on positive and end on negative charges. They represent the paths along which current will flow between charges in a resistive medium and their density reflects the local current density. The electric fields generated in a vacuum by (A) a positive monopole and (B) a dipole are shown in two-dimensions but exist in three dimensions and may also change over time with the strength and polarity of the source. (C) The electric organ discharge (EOD) of the weakly electric fish *Eigenmannia* approximates a dipole that alternates polarity through the course of each discharge. (D) The complicated pattern of isopotential lines around an anesthetized catfish result from multiple current sources and sinks, including the mouth, gills, anus and skin surface (potentials relative to a distant ground are indicated in μV; modified from Peters et al. 1974).

Imagine the unit test charge at an infinite distance from the source of an electric field. From Coulomb's Law we see that there is no force exerted on the test charge. However, if we wish to move the test charge closer to the source of the electrical field, the force acting on the test charge increases and work, either positive or negative, must be done to overcome this force. The work required to move a unit charge from an infinite distance to a given position in the electric field is called the 'absolute potential' in analogy with the 'potential energy' associated with the gravitational force. It is usually given in volts and, as an integral of the electric field over distance, the potential diminishes with distance as $1/r$. Often surfaces of equipotential are drawn and these are normal to the field lines of electrical force. No work is required to move a charge along an equipotential surface, but work equal to the difference in potentials is required to move between surfaces.

Biological systems typically respond not to the absolute potential (a reference at essentially infinite distance often being unavailable) but rather to the potential difference between two points in space. The potential difference is the spatial derivative of the potential and thus changes as $1/r^2$.

What we have described thus far is a monopole, a single charge, and seldom exists in natural systems. A common occurrence, however, is the dipole - two equal but opposite charges with some distance (d) between them (Fig. 2B). The electric field created by a dipole is a function of d and the magnitude of the charges (q):

$$E = \frac{1}{4\pi\varepsilon_0} \frac{qd\cos(\theta)}{r^3} \qquad (3)$$

The quantity, qd, is known as the 'dipole moment' stemming from the tendency of the dipole to rotate to align itself in an applied electric field. The cosine term in equation #3 indicates that the field varies with the angle relative to a line between the two charges, being maximal on the line and minimal perpendicular to it. Both the strength of the field and the potential difference drop off as $1/r^3$. In nature, fields are rarely purely dipolar (Fig. 2C) often having higher order structure as quadrapoles and octapoles and so on (Fig. 2D). The electric fields of these structures fall off more rapidly still, as $1/r^4$ and $1/r^5$, respectively, and are of importance only at very short distances from the source.

Conductors are materials that, although electrically neutral, possess a large number of mobile charges. When an electric field is applied, the electrons within the conductor move in a coordinated fashion referred to as 'electric current.' The current-density (J measured in Amps m^{-2}) is proportional to the applied electric field by Ohm's law:

$$J = \sigma E, \qquad (4)$$

where σ represents the conductivity of the substance (in Siemens m^{-1}). Resistivity (ρ) is the inverse of conductivity and is measured in units of Ohm meters[4].

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[4]Conductivity is sometimes expressed in units of $(Ohm\ m)^{-1}$

Resistance (R) to current flow is defined as the resistivity of the material integrated over the surface area and length through which the current must flow. Current (I) flowing through a surface of finite resistance is equal to the current density integrated over the cross sectional area. Thus Ohm's law can be re-expressed as:

$$I = V/R. \tag{5}$$

Note that by convention, electric current is defined as the net positive charge passing per unit time (dQ/dt) and thus flows from positive to negative charges, the opposite of the flow of electrons. For a dipolar supply of continuous current in a resistive medium, we can replace the charge and permittivity of equation #3 with ρ and I. The electric field at any point in space is given by:

$$E = \frac{\rho I}{4\pi} \frac{d \cos(\theta)}{r^3} \tag{6}$$

In contrast to a conductor, the charges (protons and electrons) in a dielectric are not mobile. Some molecules, such as H_2O, are polar because the charges are (more-or-less) bound to opposite sides of the molecule. In others, this polarization develops as a consequence of an applied electric field. The multitude of tiny dipoles that make up a dielectric align themselves anti-parallel to the imposed field. That is, their positive poles are oriented towards the negative side of the applied field and vice-versa. This anti-parallel alignment diminishes the electric field within the dielectric in proportion to the degree of polarization. The extent to which a material will polarize and impede an imposed electric field is expressed as a 'dielectric constant', k.

Water is actually both a conductor and a dielectric. Some ions are polarized and build up a counter field, while other ions are dissociated and will flow along field lines as electrical current through a resistance. A continuously unidirectional flow of ions is termed a 'direct current.' If instead, the current alternates polarity back and forth, both the free charges and the dipoles of the dielectric change direction with each change in current polarity. Atomic and molecular forces prevent these changes from being instantaneous and as the frequency of current alternation increases, the dipole realignment begins to lag and the counter field impedance decreases. Thus, in actuality, the dielectric acts as a high pass filter and the 'dielectric constant' is not constant at all, but rather depends upon the frequency of the applied alternating field. The overall *impedance* of the water to an alternating field will thus be a function of the water's resistivity and the counter-field induced at the frequency of alternation.

Cell membranes display another electrical property in that they can store charge. If a voltage is applied across two parallel conducting plates that are separated by a small distance (as is true for a cell membrane), positive charges build up on one plate and negative charges on the other. The amount of positive charge built up per volt is the 'capacitance' (C):

$$C = \frac{Q}{V} \text{ or } Q = CV \tag{7}$$

Current (the flow of charge per unit time) will flow across a capacitor only when the voltage is changing and in proportion to the rate of change:

$$I = \frac{dQ}{dt} = C\frac{dV}{dt} \tag{8}$$

A current that alternates with a given frequency, ω[5], may be altered in phase and in amplitude as it flows across the capacitor:

$$I = C\omega V \cos(\omega t) \tag{9}$$

If we examine the amplitude term only, $V/(1/C\omega)$, we see that the resistive impedance of a capacitor is inversely proportional to the product of the frequency and capacitance:

$$R = \frac{1}{\omega C} = \frac{1}{2\pi f t C} \tag{10}$$

Finally, we note that most biological capacitors lie in parallel with resistive elements (R). As a current is applied to the system, the capacitor will charge (and then discharge) with a time constant[6] equal to the product of RC.

EVOLUTION AND DISTRIBUTION OF ELECTRORECEPTION

Specialized sensory organs and associated brain structures to detect weak electric fields probably initially evolved as a primitive trait of the vertebrates (Bullock et al. 1983; New 1997). Electroreception persists in all extant non-teleost fish taxa except the hagfish (Myxiniformes), and gars and bowfins (Holostei). Two of three orders of amphibian are electroreceptive; the salamanders and caecilians, but not the anurans. The electric sense seems to have been lost to the ancestral form that gave rise to the gars, bowfins and teleosts but later was newly evolved twice amongst the teleosts, in the osteoglossiforms (notopterids, mormyriforms) and in the siluriform/gymnotiform (catfish and knifefish) lineage. Each of these origins is closely allied with and probably derives from the lateral line mechanosensory system. Recently, the semi-aquatic echidna and platypus have been shown to have independently evolved electroreception for a fourth time, but this time from a trigeminal precursor (Andres et al. 1988; Manger and Hughes 1992).

Several taxa have independently evolved specialized electric organs (EOs) for producing electric fields to serve a variety of functions. A few species, most notably the gymnotiform electric eel, *Electrophorus*, produce high voltage shocks, of tens to hundreds of volts, for both defense and predation. Other species, including some skates and rays, the stargazer (*Astroscopus*), the African mormyriforms, the synodontid and clariid catfish (Hagedorn et al. 1990; Baron et al. 1994a,b), and the remaining South American Gymnotiforms, produce only

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[5]Angular velocity ω, is equal to the product, $2\pi f t$, where f is the frequency and t is time.

[6]The time constant represents the time to charge to $(1-1/e)$, about 63%, of the final value.

weak electric fields, on the order of a few hundred millivolts to a few volts peak-to-peak when measured from head to tail. Within a species, electric organ discharges (EODs) generally describe a stereotypical waveform that may show small differences between sexes and/or seasonally and even between individuals. In some species, termed 'pulse-type' species, EOD pulses are quite short (a few ms) and stereotyped relative to a variable and longer inter-pulse interval. Individual pulses are spectrally broad. Pulse-type species include all the mormyrids and many gymnotiforms. The EODs of apteronotid, eigenmanniid and sternopygid gymnotiforms and the mormyriform *Gymnarchus* are more sinusoidal ('wave-type') in nature with a strong fundamental frequency and several harmonics.

ANATOMY AND PHYSIOLOGY OF ELECTRORECEPTORS [7]

Students of shark anatomy are familiar with the many pores dotting the skin surface across the head region of the shark and leading to jelly-filled canals. These are the external signs of the ampullary electroreceptor organs, the 'ampullae of Lorenzini,' and exemplify the general electroreceptor form consisting of an epidermal inpocketing that forms a pore and canal and broadens into a chamber, or ampulla, lined by a sensory epithelium (Fig. 3). A low impedance canal (R_{canal}) coupled with a high impedance canal wall (C_{wall}, R_{wall}) ensures that the chamber's lumen and the pore opening to the external environment are isopotential. Receptor cells within the sensory epithelium act as voltage to chemical transducers (*n.b.* the receptor to afferent synapse may be electrical in *Apteronotus*) and are positioned to detect a potential difference between the lumen and the local interior of the fish. The sensory receptor cells are connected by tight junctions to neighboring supporting cells and these high-resistance junctions effectively isolate the receptor cell surface into a basal face that is electrically close to the internal milieu of the fish and an apical face that contacts the lumen of the chamber (Fig. 3). A lumen-negative stimulus will cause an outward current through the receptor cell's apical face (depolarization) and an inward current through the basal face (hyperpolarization). A lumen-positive stimulus elicits the opposite polarity response. Unlike the lateral line and auditory systems there are no efferent connections to electroreceptors. Receptor cells make synaptic contact with afferent nerve fibers that carry information into the central nervous system. The first synaptic target in the brain is the dorsal octavolateral nucleus of non-teleosts or the electrosensory lateral line lobe (ELL) of teleosts.

Electroreceptor organs of fishes can be categorized functionally into two types according to the stimulus frequencies to which they are most responsive. Electroreceptors found in the ancestral lineage comprise a pit or canal with a pore opening to the outside, thus giving them an ampule-like shape and earning the designation of "ampullary" organs. Ampullary electroreceptors respond to low frequency electrical stimuli (near d.c. to several tens of Hz) with an up- or down-modulation of their ongoing tonic rate of discharge. Similar, but newly evolved low-frequency receptor organs within the teleosts are also called 'ampullary' (Fig. 3,

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[7]A more detailed treatment of electroreceptor anatomy and physiology may be obtained from the several reviews contained within Bullock and Heiligenberg (1986)

top). As will be discussed below, ampullary organs are more closely similar within habitat than they are taxonomically. The second functional class of receptor organ, while diverse in appearance, typically have sensory receptor cells that protrude into the lumen of the canal, thus earning their name as 'tuberous' electroreceptor organs (Fig. 3, bottom). Tuberous electroreceptors are known from the mormyriform and gymnotiform electric fishes where they serve for electrolocation and communication. They are responsive to high frequency stimulation, generally near to peak spectral frequencies of the fish's own EOD (fEOD), and are continuously driven by the EOD. Numerous subtypes of tuberous organs have been identified and will be discussed below. Tuberous electroreceptors have also been described anatomically from the blind cave catfish (Andres et al. 1988).

AMPULLARY ELECTRORECEPTORS

The ampullary system is exquisitely sensitive to small potential differences and is used for detection of prey and possibly for orientation within standing electromagnetic fields and for communication. The thresholds for primary afferents of marine species can be less than a microvolt (measured at the pore relative to a distant reference), whereas those for freshwater species are on the order of tens to hundreds of microvolts. Behavioral thresholds for several species of shark or ray are less than 0.01 μVcm^{-1} (at 0.5 $nAcm^{-2}$; Kalmijn 1982), for the freshwater catfish, *Ictalurus*, about 2 μVcm^{-1} (at 0.8 $nAcm^{-2}$; Peters and van Wijland 1974) and for the weakly electric fish, *Sternopygus*, about 5 μVcm^{-1} (at 2.5 $nAcm^{-2}$; Kalmijn 1974). A number of specializations appear designed to allow this sensitivity (Fig. 3, top). The typical elasmobranch ampullary canal ends in a cluster of small bulbous sacs, each lined by the apical surfaces of hundreds of receptor cells and their adjoining support cells. Freshwater species have only a single ampulla and a much smaller number of receptor cells. In contrast to the isopotential canal, there is an almost uniform potential drop across the fish's skin and internal tissues. A receptor cell thus experiences the potential difference existing between the canal pore and the fish's internal milieu near to the ampullary sacs.

Each receptor cell makes 20-30 deeply invaginated ribbon synapses with several afferent nerve fibers (Fields and Ellisman, 1985) and each afferent fiber makes contact with many tens of receptor cells. A constant 'bias' current keeps the receptor cells depolarized and releasing transmitter. Primary afferents thus discharge at rest with a fairly constant rate between about 20 and 60 Hz and with a uniform interspike interval. In elasmobranchs, both faces of the receptor cell are active but the conductance change in the apical face is far greater. A lumen negative (cathodal) stimulus will cause a large increase in the outward current across the apical face and depolarizes the receptor cell. This in turn causes an increase in transmitter release and a concomitant increase in primary afferent firing. A lumen positive (anodal) stimulus has the opposite effect and results in a decrease of afferent firing. In teleosts (Fig. 3 top), only the basal face of the ampullary receptor cell is active. Lumen negative stimuli cause a large increase in the inward current across the basal face and hyperpolarize the cell. Thus, in teleosts, afferent firing is decreased by lumen negative stimuli and increased by lumen positive stimuli.
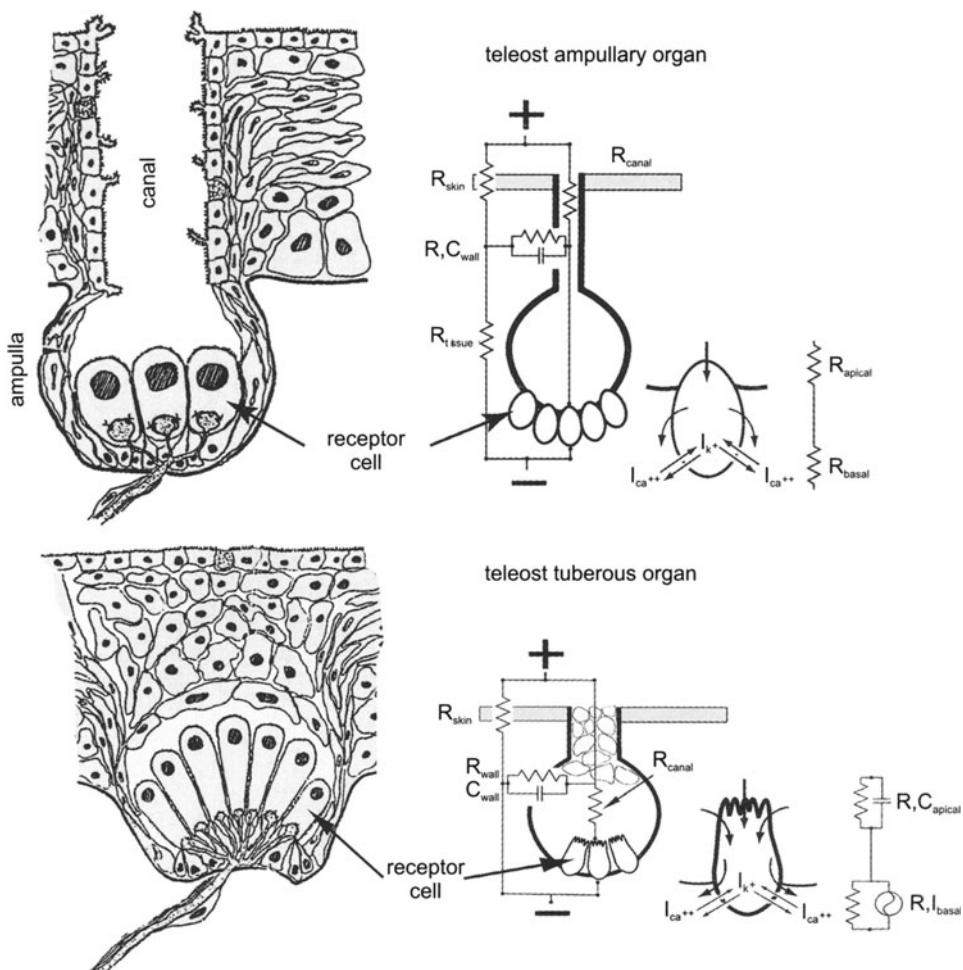
Fig. 3 Diagrammatic drawings and equivalent circuits for teleost electroreceptor organs and their sensory receptor cells (right). The 100-200 μm long canal of a teleost ampullary organ (top) connects the outside seawater milieu with a subdermal chamber, or ampulla, which is lined with a sensory epithelium. The resistance along the canal (R_{canal}) is much less than the resistance across the canal wall (R_{wall}) so that current is shunted from the pore to the receptor cell with only some high frequency loss through a high wall capacitance (C_{wall}). Outside *positive* stimuli cause current (thick arrows) to flow inward across the receptor cell's apical membrane (R_{apical}). This depolarizes the receptor cell causing calcium channels to open in the basal face (allowing I_{Ca++}), which further depolarize the cell and cause the release of neurotransmitter. A delayed, calcium-dependent potassium current (I_{K+}) repolarizes the cell. An outside *negative* stimulus has the opposite effects, causing the receptor cell to hyperpolarize and lessening its release of transmitter. Gymnotiform tuberous receptors (bottom) show several important differences. High frequency loss across the short canal wall is minimized by a low C_{wall}. Tuberous receptor cells protrude into the lumen, presenting a large apical surface area (low R_{apical}, high C_{apical}). A lumen positive stimulus will cause current to flow inwards (I_{Ca++}) across the apical surface to depolarize the cell, but only at frequencies that are high enough to pass the membrane's capacitance. Bandpass tuning is probably enhanced by the resonance of a depolarizing inward I_{Ca++} and repolarizing outward I_{K+} on the basal face (R_{basal}, I_{basal}). (Adapted from Heiligenberg 1993; Bennett 1967; and Bastian 1994)

Skin resistivities of marine and freshwater species share a range from a few hundred to about ten thousand Ωcm^2 (*n.b.* Bennett 1965). The average resistance across the skin and body of a marine elasmobranch is about two orders of magnitude greater than that of an equivalent volume of seawater ($\rho_{seawater} = 20\text{-}26\ \Omega\text{cm}$, Kalmijn 1987) and thus the fish's body acts as an insulator within the seawater. In a uniform electric field, about half the voltage drop across this insulator will occur across the skin. The remaining voltage drop is uniformly distributed across the internal tissues and thus the voltage difference relative to outside the fish will depend upon where the internal measurement is made. In marine species, the receptor-cell containing ampullae are organized into a small number of distinct clusters on the head that each allow for a common internal reference potential. Canals extend across the head and pectoral fins in many directions in a species specific pattern allowing the receptor cells of each ampulla to sample the potential from distinct skin regions (Tricas and Sisneros, this volume). Electroreceptors are sensitive to the component of the electric field that is parallel to the canal. Thus, in a uniform electric field, the potential difference across the receptor cell will be determined by the length of the canal and by the cosine of the angle between the external electric field and a line from the pore to the receptor cell. In contrast to a uniform field, the localized fields of small dipoles provide only transepidermal voltage gradients. There is little additional voltage drop across the internal tissues and canal length will have little effect on the strength of these potential differences.

Freshwater species may be somewhat more resistive than marine species, but are relatively less resistive than their freshwater environment ($\rho_{freshwater} \sim 2\text{-}100\ \text{k}\Omega\text{cm}$ in the Amazon basin) and their body acts as a current sink. For a uniform field, the voltage drop across the fish is proportionally less than in saltwater. Freshwater species have short canals (100-200 μm) that penetrate only the epidermis and allow voltage comparisons between the exterior and a nearly isopotential internal milieu. Recent measurements and modeling suggest a complex picture of relatively low and inhomogeneous internal and skin resistivities that support variable shunting of currents and differential concentration of voltage drops to certain areas of the skin. For example, the dorsal and ventral skin surfaces, but not the flank of *Gnathonemus* are richly endowed with electroreceptors and have an extra outer layer of epidermal cells that raises the local skin resistivity by about an order of magnitude (Caputi et al. 1998).

TUBEROUS ORGANS OF TELEOST FISHES

Tuberous electroreceptors serve both communication and electrolocation. They are typically band-pass tuned near to the peak power frequencies of the fish's own EOD or that of their conspecifics. Although there is a wide variety of morphological types, the prototypical tuberous organ comprises a short canal from the skin surface that swells into a chamber into which protrude 20-30 receptor cells (Fig. 3, bottom). A loose cellular cap fills the pore. Several mechanisms probably contribute to the band-pass tuning of tuberous receptors. Shunting of high frequencies across the walls of the chamber and canal is minimized by many thin layers of epithelial cells that together present a low capacitance. The protruding apical face of each receptor cell comprises a highly proliferated cell membrane that serves as a series blocking

capacitor to assure passage of only voltage changes that are not slow relative to the membrane time constant. The receptor cell's basal face has active conductances (probably K^+ and Ca^{++}, Zakon 1984) that cause voltage oscillations near to the best frequency for stimulation. The synaptic connections with afferent fibers and the fiber specializations are quite diverse but typically reflect a convergence of most or all the receptor cells from a single chamber onto a single afferent fiber with a large number of ribbon synapses from each receptor cell.

Many of the morphological and physiological specializations of the tuberous system can be understood in terms of specialization for preserving and encoding either *time* or *intensity* information about high-frequency electrical stimuli. Separate central nervous system pathways served by different types of tuberous organ are often highly adapted to further enhance and refine the encoding of either time or intensity cues. Time-coding pathways mark the time or phase of each EOD with high reliability and fidelity over a broad range of stimulus intensities. Even at relatively high stimulus frequencies (ca. 2 kHz in *Apteronotus spp*) phase-locking to the EOD is possible. Convergence of afferent input from multiple receptors onto higher level cells may help reduce the jitter of time-encoding but may also limit the spatial resolution of timing systems. Specializations to minimize, standardize and compensate for axonal and synaptic conduction delays may include large diameter fibers with minimal branching and enlarged synaptic terminals and/or electrical synapses. Highly specialized circuitry is known from the gymnotiform *Eigenmannia* that allows discrimination of time differences as small as 0.4 μs between different areas of skin surface.

Sensitivity to spatial and temporal patterns of stimulus intensity give rise to a different set of specializations. Intensity-coding electroreceptors often respond more vigorously and with shorter latency to stronger stimuli. Primary afferents are often more broadly tuned (Hopkins 1981; Zakon 1986), perhaps to fully encode the power of a broadband stimulus or to avoid potential non-linearities often associated with narrow tuning. Intensity-coding afferents are also usually tuned below the peak spectral frequencies of the fish's EODs. Within the central nervous system, fine-grained and sometimes multiple somatotopic maps enable lateral inhibitory and descending networks to modulate local excitability and improve spatial contrast.

The tuberous systems of gymnotiforms and mormyriforms offer differing variations on these common themes.

Gymnotiform Tuberous Electroreceptors

Tuberous receptors of gymnotiforms morphologically resemble the prototype described above. Tuberous afferents form a bimodal distribution of response types from broad tuning coupled with relatively low best-frequency compared to sharper tuning curves centered at higher frequencies (Fig. 4A). The time-coding system is served by the more sharply and higher tuned afferents variously known as 'T-units' (for 'timing') in wave-type species and 'pulse-markers' in pulse-type fishes. These afferents receive input from 3-7 receptor organs in adult *Sternopygus* (Sanchez and Zakon 1990). They typically fire a single spike phase-locked to the EOD with low jitter. Timing units have low absolute thresholds (ca. 300 μV cm^{-1} relative to a distant ground) and their responses are non-adapting.
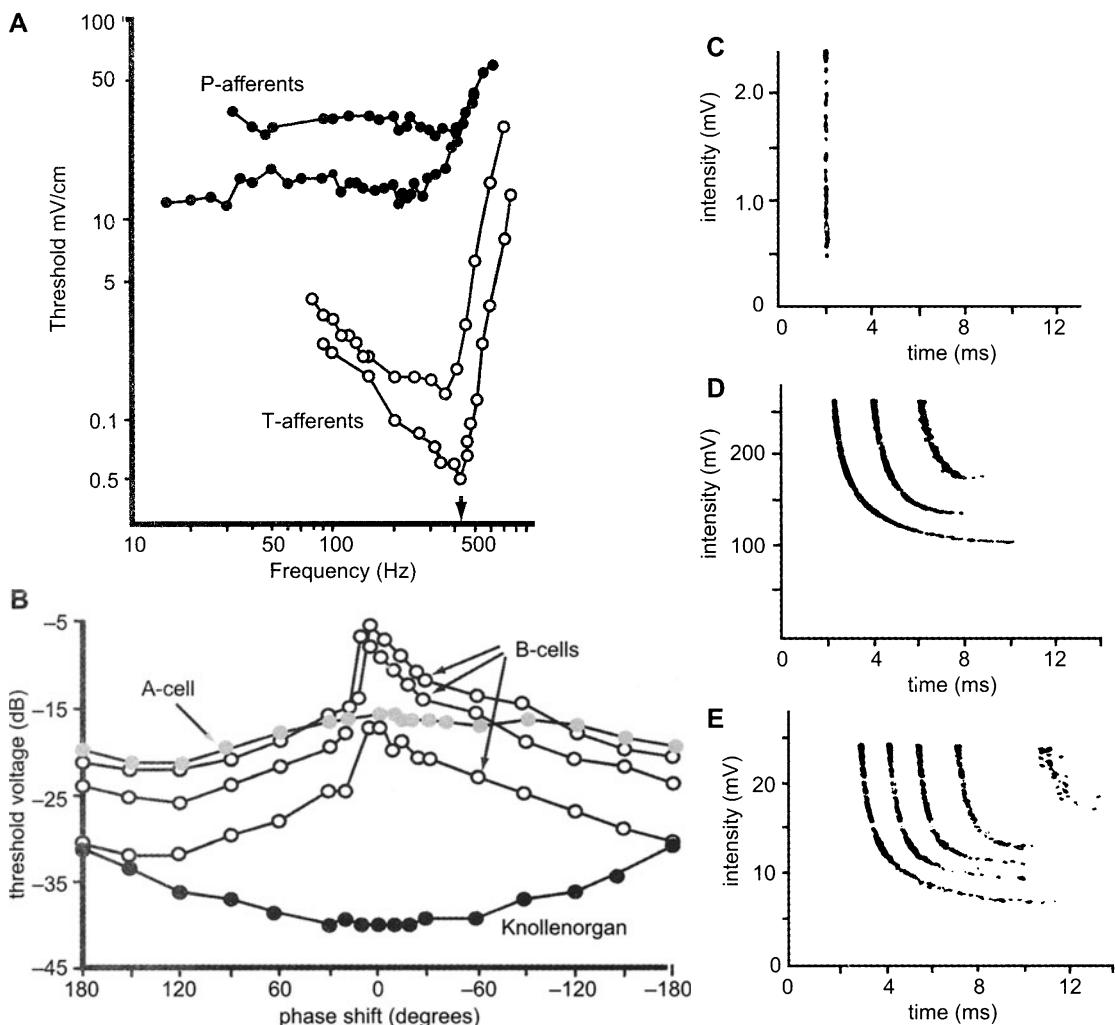
Fig. 4 Representative response properties of tuberous electroreceptors. (A) Frequency response curves for four cells obtained from an individual *Eigenmannia*. Higher threshold 'P-type' afferents are broadly tuned to frequencies at and below the EOD frequency (f_{EOD} = 428 Hz, arrow). Their probability of firing depends on the stimulus amplitude. More sensitive 'T-type' afferents phase lock to the EOD and are sharply tuned near to the fEOD. Modified from Hopkins (1976). (B) Primary afferents from tuberous electroreceptors of the mormyrid, *Gnathonemus petersii*, respond to EODs that have been phase-shifted. A 0° phase shift is the normal EOD, ±180° phase shifts are inverted. The low-threshold knollenorgans and mormyromast 'A-type' afferents show little change in threshold to phase shifted EODs. The threshold of mormyromast 'B-type' cells, however, is elevated by 10 to 15 dB when the waveform is most similar to the normal EOD and are, thus, inversely waveform tuned. Modified from von der Emde and Bleckmann (1997). (C - E) Raster displays of the responses of *G petersii* afferents show the effects of stimulus intensity. The stimulus was a 15 ms outside-positive square wave. (C) Knollenorgans fire a single, constant latency spike at low threshold. The multi-spike responses of mormyromast (D) 'A-type' and (E) 'B-type' cells, however, decrease smoothly as stimulus intensity is increased. Note the differences in ordinate scales. Modified from Bell (1990).

Stimulus intensity is encoded in the response of the more broadly tuned subclass of afferents, the 'P-units' (for 'probability') of wave-type species and the 'burst-duration coders' of pulse-type species. The probability and/or number of spikes for these units depends on the stimulus intensity. These afferents receive input from only 1 or 2 receptor organs. Their absolute thresholds are generally higher than those in the timing pathway (ca. 1.8 mVcm^{-1}) and their responses adapt to a changed intensity over time.

Tuberous receptors are distributed non-uniformly across the skin surface. In *Apteronotus*, for example, concentrations are highest on the snout (20-25 mm^{-2}) and face (15 mm^{-2}) falling off to about 3 mm^{-2} on the trunk (Carr et al. 1982). In pulse-type gymnotiforms, systematic differences along the length of the fish in the magnitude and orientation of current flow across the skin lead to differences in the local EOD spectrum and correlated variation in the tuning of electroreceptors both to frequency and to the orientation of the electric field vector (Bastian 1977; Yager and Hopkins 1993).

Mormyrid Tuberous Electroreceptors

The mormyrid knollenorgans are time-coding tuberous electroreceptors used for social communication (Bell 1989). They rapidly adapt and fire a single spike at a fixed latency to each EOD-like stimulus (Fig. 4C). The primary afferent threshold is very low, about 0.1 mV across the skin, and there is only a small latency decrease as the stimulus level is increased. Knollenorgan primary afferents are adapted for high transmission speeds; they have large diameter myelinated axons, with few branches and mixed chemical-electrical synapses give rise to rapid and large epsp's within their target cells of the nucleus of the ELL. Knollenorgans are strongly driven by EOD-like stimuli that contain large and rapid transients and, of course, respond well to the fish's own EODs. Within the nucleus of the ELL, however, the knollenorgan system receives a brief and precisely timed inhibition as a corollary discharge from the EOD-command pathway. This inhibition eliminates the knollenorgan response to the reafferent EOD. Spatial information is only weakly maintained within the knollenorgan system with little somatotopy and high levels of convergence.

The mormyromast system is specialized for encoding the spatial distribution of stimulus intensity that is crucial for active electrolocation and mormyromasts outnumber knollenorgans by about ten to one. Near threshold, mormyromast afferents fire a single spike at latencies of 9-12 ms (Fig. 4D,E). As the local stimulus intensity increases additional spikes may be added and the first spike latency decreases in a smoothly graded manner to 3-4 ms. Thus mormyromasts encode stimulus intensity and not stimulus timing in the timing of their spikes. Mormyromast electroreceptors are unique in that they have two sensory chambers, an outer and an inner, each with their own receptor cell type, A and B, respectively, that represent different submodalities (Bell 1990; von der Emde and Bleckmann 1997). In *Gnathonemus*, type A cells are relatively high threshold amplitude coders (note the y-axis scale in Fig. 4D). Their small and variable apical surface area correlates with a wide range of thresholds that may increase the population's ability to encode a wide range of stimulus intensities. Type B thresholds are generally lower, resulting in part from an elaborated apical face, which may lower its

resistance, and an oscillatory receptor potential on the cell's basal face. Type B cells are sensitive to the shape of the local EOD waveform, showing a markedly lower threshold to phase-shifted waveforms (Fig. 4B). The two types project to differing zones within the ELL for further processing in a highly organized and somatotopic fashion. As in the knollenorgan system, electrotonic synapses onto secondary neurons within the ELL preserve the time code. In contrast, however, excitatory receptive fields are small and there is a well-developed system of lateral inhibition. Mormyromast secondary neurons receive a brief, time-locked excitatory corollary discharge that may serve to enhance responsiveness to the fish's own EOD and perhaps also serve as a timing reference (Bell 1989).

PASSIVE ELECTROLOCATION

Sharks and rays can localize their prey using their exquisitely sensitive ampullary system. Kalmijn (1978) lured the shark, *Scyliorhinus canicula*, near to a buried flounder and then discovered that the shark would orient to and strike at the flounder even when any mechanical, chemical or visual cues had been eliminated by covering the prey with an agar box. The only remaining cue was the weak 'bio-electric' signal given off by the flounder. In a subsequent trial, sharks attacked a buried pair of electrodes that emitted dipole electrical fields having frequencies from 0 to 8 Hz. Direct current voltage gradients as small as 5 $nVcm^{-1}$ were sufficient in later studies (Kalmijn 1982) to elicit orienting and strike responses from the dogfish, *Mustelus canis*, the blue shark, *Prionace glauca*, and the sting ray, *Urolophus halleri*.

How is such remarkable sensitivity of the ampullary system achieved? Several mechanisms can be identified. First, longer ampullary canals allow greater sensitivity to uniform (large scale) electric fields. In addition, comparison of signals from opposite sides of the body may double the effective voltage difference. Secondly, modulation of an ongoing basal level of transmitter release from receptor cells may allow greater sensitivity than would the de-novo activation of sub-threshold cells. The high regularity of resting discharge may facilitate even small modulations in firing rate to be detected by more central neurons. While the highest sensitivity is found near threshold, primary afferents adapt to sustained d.c. levels with a time course of tens of seconds to maintain a relatively high sensitivity across a wide range of stimulus levels (Murray 1965; Bodznick et al. 1993). An improvement in signal to noise of nearly one order of magnitude ($\sqrt{n}$) may result from the convergent input onto primary afferents from many tens of receptor cells in a single ampulla. Although the timing of spontaneous transmitter release from each receptor cell within a given ampulla is likely to be independent and random, the stimulus-induced modulation of release should be nearly identical in both phase and amplitude for each receptor cell. Additional convergence and concomitant noise reduction is likely at higher levels of sensory processing.

Such extraordinary sensitivity suggests that ampullary organs respond to many other potential stimuli in addition to the bioelectric fields of their prospective prey (Montgomery and Bodznick 1999). Indeed, ampullary receptors probably respond to noise from all the potential sources discussed earlier as well as to stimulation by chemical, mechanical and temperature modalities. Two of the most potent sources of noise are mechanical stimulation from the

animal's own movements and the animal's own bioelectric field. Self-motion induced mechanical stimulation may be minimized by adopting a 'sit and wait' style of predation or by minimizing and perhaps stereotyping body movements while moving about. Skates may 'walk' about on modified pectoral fins, while rays roam the bottom using only small movements of the tips of the pectoral fins. Perhaps as a further adaptation to reduce mechanically induced noise, the ampullary canals of the ray extend across only the rigid portion of the fins and not all the way into their more flexible tip (Tricas and Sisneros, this volume).

A first stage of filtering occurs with the tuning properties of ampullary receptors. The ampullary canal serves as a low-impedance shunt, but longer canals attenuate higher frequencies more strongly (Waltman 1966). At canal lengths less than about 10 cm, the tuning properties of the receptor cells themselves determine the response characteristics. Although ampullary receptors are basically 'low-pass' and respond to very low frequency stimuli, the cell membrane's capacitance precludes a response to the ongoing d.c. level. The fish's own d.c. bioelectric field is modulated at low frequencies by ventilatory movements of the mouth, gill slits and/or spiracles (Bodznick et al. 1993). The field strength is quite variable, albeit slowly, over time and space, but is modulated synchronously across all the electroreceptors and is thus 'common-mode.' The ventilatory modulation falls well within the tuning of the electroreceptors and is represented in the activity of primary afferents (Fig. 5A). Secondary neurons, the so-called principal cells of the dorsal nucleus, however, show an almost five-fold average improvement from the primary afferents in their response to a local dipole test-source versus the common-mode ventilatory rhythm (Fig. 5B). This improvement arises in part by the elimination of signals held in common across excitatory and inhibitory receptive fields of secondary neurons. A second and more powerful technique for noise elimination is an adaptive filter that 'learns' to ignore stimuli that are consistently time-locked to the ventilatory rhythm. Such a mechanism was first and most completely described by Curtis Bell and his colleagues for the negation of EOD-locked stimuli from cells in the ELL of the weakly electric mormyrid fishes (Bell et al. 1999). Very similar mechanisms have now been found to operate in the ELL of gymnotiforms (Bastian 1995) and the dorsal nucleus of elasmobranchs (Bodznick et al. 1999). The elasmobranch secondary neurons receive extensive independent cancellation signals from motor commands, proprioceptive signals and descending electroreceptive (common-mode) input. The synaptic weights of these inhibitory inputs are adjusted over minutes to create a 'negative image' (Bell 1989) of stimuli that are time-locked to the ventilatory cycle. The negative image is quite specific, depending on the time in the cycle as well as the spatial location and polarity of the stimulus and, in some neurons, can completely eliminate self-generated re-afferent activity (Fig. 5B).

Planktonic *Daphnia* produce a dipolar d.c. electrical field of up to 1 mV at 1 mm distance. Feeding and swimming movements of their appendages modulate the field at low frequency (3-15 Hz). Lon Wilkens and his collaborators (Wilkens et al. 2001; Wilkens, this volume) demonstrated that paddlefish (*Polyodon spathula*) can localize and discriminate between individual *Daphnia* by these weak bioelectrical signals. This is a very short distance behavior, however. Average capture distances are about 1.3 cm and detection occurs out to about 4 cm. The basal firing rate of an electrosensory primary afferent is modulated by a *Daphnia* when it passes within a only few millimeters of the receptive field center.

A primary afferent

B secondary electrosensory neuron

1 s
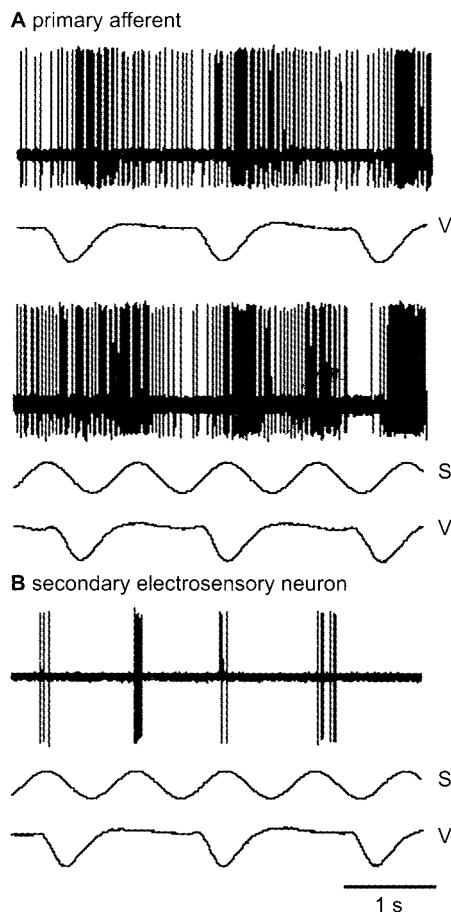
Fig. 5 In the electrosensory system of the skate, reafferent activity that is time-locked to the ventilatory rhythm is selectively eliminated in second order neurons. (A) Primary afferents are strongly driven by the ventilatory modulation (V) of the skate's standing bioelectric field (top). The response to a sinusoidal dipole stimulus (S, bottom, 2 mV peak-to-peak relative to a distant ground) is largely hidden within the ventilatory response. (B) Principal neurons of the dorsal nucleus are subject to an adaptive cancellation filter that eliminates the reafferent response and reveals the response to the dipole stimulus. Modified from Bodznick et al. (1999).

The skin of a catfish is liberally speckled with thousands of ampullary organs that could be used for detecting and orienting to nearby prey (Peters et al. 1974; Kalmijn 1974). Piscivorous catfish detect the bioelectric fields of other fish as well as the near d.c. components present in EODs of weakly electric fishes (Lissmann and Machin 1963; Peters and Buwalda 1972). Many of the larger neotropical pimelodid catfish consume gymnotiforms opportunistically (Reid 1983) while at least one species, *Pseudoplatystoma tigrinum*, is a gymnotiform specialist. In contrast to many of the wave-type species, pulse-type gymnotiforms have spectrally broad EODs with significant low frequency energy and, perhaps not coincidentally, are most often found in smaller streams away from the main river channels where these catfish are found. In southern

Africa, packs of migrating clariid catfish hunt mormyrids as they leave the draining flood plains for the main river channels (Merron 1993; Hanika and Kramer 2000). The catfish appear to specialize on male *Marcusenius macrolepidotus*, whose long-duration EODs have significant low frequency energy. Other sympatric mormyrid species as well as female *M. macrolepidotus* lack the low frequency energy and are not significant components of the catfish diet. Examples such as these have prompted Phil Stoddard (1999) and Mary Hagedorn (personal communication) to suggest that predation has been a strong selective force to lessen the d.c. component present in EODs.

Behavioral thresholds for detection by freshwater catfish are on the order of 1 to 10 μVcm^{-1} resulting in threshold distances of a meter or two. The organization of first order electrosensory processing (McCreery 1977a,b) suggests that many of the same noise suppression and feature extraction mechanisms are at work in the catfish as in the tuberous system of the closely related (and more extensively studied) gymnotiforms. One result is a somatotopic mapping of electrosensory space within the midbrain (Knudsen 1976) that might facilitate electrolocation and identification.

Neither bio-electrical signals of prey nor the EODs of weakly electric fish present unambiguous vectorial cues that point towards the source of the electric field. The curving horizontal electric field lines provide a reliable if less direct route to either pole of the dipolar field (Fig. 2B). Carl Hopkins and his colleagues have demonstrated that both gymnotiforms (*Gymnotus*, *Brachyhypopomus*) and mormyrids (*Brienomyrus*) approach dipole sources of artificial EODs by swimming in alignment with the field lines with zero average offset (Fig. 6). Only when the source was at very close range (approaching a near-field condition) did the fish make a more direct approach. The fish showed a slight bias to approach the positive pole preferentially, which might reflect a preference for the initially head-positive EOD. Kalmijn (1987) has suggested a similar algorithm whereby sharks might approach a dipole field with a constant non-zero orientation to the electric field lines.

Hopkins and colleagues (Schluger and Hopkins 1987) suggest a number of possible neuronal strategies that might allow the fish to follow electric field lines. Ampullary and tuberous receptors are most sensitive to the component of the electric field vector that is perpendicular to the skin. Midbrain maps of vector orientation have been found in the ampullary systems of catfish (Knudsen 1976) and rays (Schweitzer 1986). If a similar map served the tuberous system of weakly electric fishes, one would expect the pattern of activity across such a map to reflect the fish's orientation within the electric field. An alternative strategy would seek to equalize the strength of stimulation on each side of the fish. When not aligned with the electric field, a turn towards the side receiving stronger stimulation will improve alignment. The time-coding system of gymnotiform fishes is specialized for comparison of signal timing across different parts of the body surface. Since the latency of pulse-marking tuberous electroreceptors indicates the strength of stimulation, a comparison of timing between the two sides could underlie proper orientation. In mormyrids, the more sensitive knollenorgan system is the most likely candidate to serve passive electrolocation. Other methods for comparison of stimulus strength across the body are, of course, possible. Another available cue involves the location on the skin of a phase
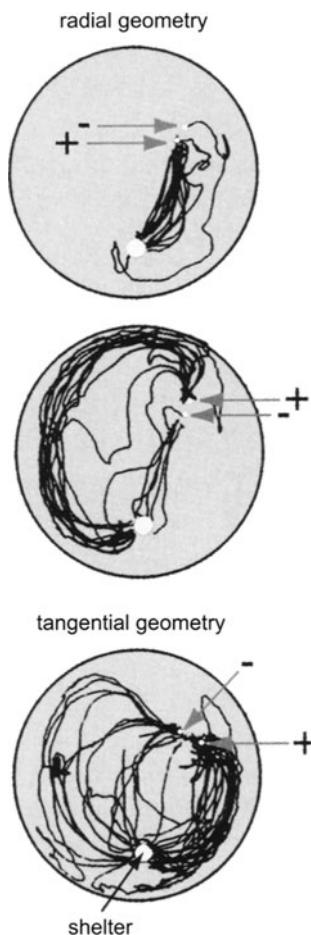
Fig. 6 Weakly electric fish approach a dipole source along the electric field lines. The pulse-type gymnotiform, *Gymnotus carapo,* was placed in a shelter (large white circle) within a larger circular tank and presented with a series of single-cycle sinusoids from a pair of electrodes near the opposite side of the tank (white dots indicated by arrows). When the stimulus was turned on, the fish responded by swimming towards and attacking the electrodes with a bias to preferentially attack the positive electrode. Tracks from individual stimulus presentations in each of three electrode geometries are shown for one fish (n = 20, 19, 36 from top to bottom). Modified from Hopkins et al. (1997).

reversal. If the fish is aligned parallel to the field lines, current will enter at the head and exit posteriorly. The resulting phase reversal will ring the body like a belt. When alignment is perpendicular, the phase reversal will fall along the midline.

Migrating sharks might be able to use their electric sense to orient to the earth's magnetic field. Kalmijn (1974) has proposed a mechanism that might allow a shark to navigate by electric currents induced as it drifts within a flow of seawater. Recently, Paulin (1995) has criticized this model, in part, due to the ambiguities arising to the fish from the complexities of the motional

electric field. Paulin suggests that active swimming through the earth's magnetic field will induce an electric current in the ampullary canals. The sharks' natural undulatory swimming pattern would thus cause ampullary afferent firing to be modulated in phase with the head rotation as the angle of the head changes with respect to the earth's magnetic field. Careful and thoughtful experimentation is still necessary to judge either of these competing hypotheses.

ACTIVE ELECTROLOCATION

The EODs of weakly electric fishes present a complex spatio-temporal pattern of transepidermal potentials along the length of the fish (Figs. 2C, 7). The gymnotiform *Eigenmannia* uses its tuberous electroreceptors to sense modulations in this pattern to facilitate its habit of daytime hovering amongst the roots and debris of floating aquatic plants. Walter Heiligenberg (1973) was able to train an *Eigenmannia* to hover between two plexiglass strips as they were swung from side-to-side. When the plexiglas was replaced with electrically invisible agar strips, the fish was unable to follow. The normal hovering behavior also deteriorated when the fish was 'jammed' by presentation of another electrical signal that mimicked the EOD of a neighboring conspecific. How is this 'active' electrolocation possible and why does the presence of a neighboring fish's EOD interfere?

Active electrolocation depends on the fish's analysis of spatial and temporal patterns of perturbations of the transepidermal potentials generated by its electric organ (Fig. 7). These patterns are called an 'electric image.' The perturbations caused by objects depend on their size, distance, shape and electrical properties and can be used by the fish to locate and identify or to align themselves relative to the object. Additional keys to understanding the electric image include a description of the generation and form of the fish's local EOD as well as of the behavioral strategies the fish might use for presenting the EOD to an object of interest.

Behavioral Strategies

The behavioral strategies employed during electrolocation are diverse. Some species probe the sediments with an electroreceptor-studded 'Schnauzenorgan.' Many gymnotiforms employ their full body-length anal fin to swim straight forwards or backwards to electrically probe an object. They may hold their body fairly rigid, perhaps to minimize EOD distortions, or they may focus their EOD by curving their body around an object. Malcolm MacIver and Mark Nelson have described a stereotyped sequence of actions used by the gymnotiform *Apteronotus albifrons* to capture individual planktonic *Daphnia* (MacIver et al. 2001, Nelson and MacIver 1999). During searching, *Apteronotus* typically swims forward with the body held relatively straight and the head pitched downwards about 30°. The leading dorsal surface is covered with the highest density of electroreceptors and, in *Apteronotus*, includes a highly specialized 'dorsal filament,' which is richly endowed with time-coding tuberous receptors (Franchina and Hopkins 1996). Prey detection is marked by a reversal to backwards swimming coupled with a sidewards roll, keeping the prey dorsal of the fish while moving it closer to the fish's mouth. A mean detection distance of 2.8 cm ranging outwards to about 5 cm was observed at a water conductivity of 35
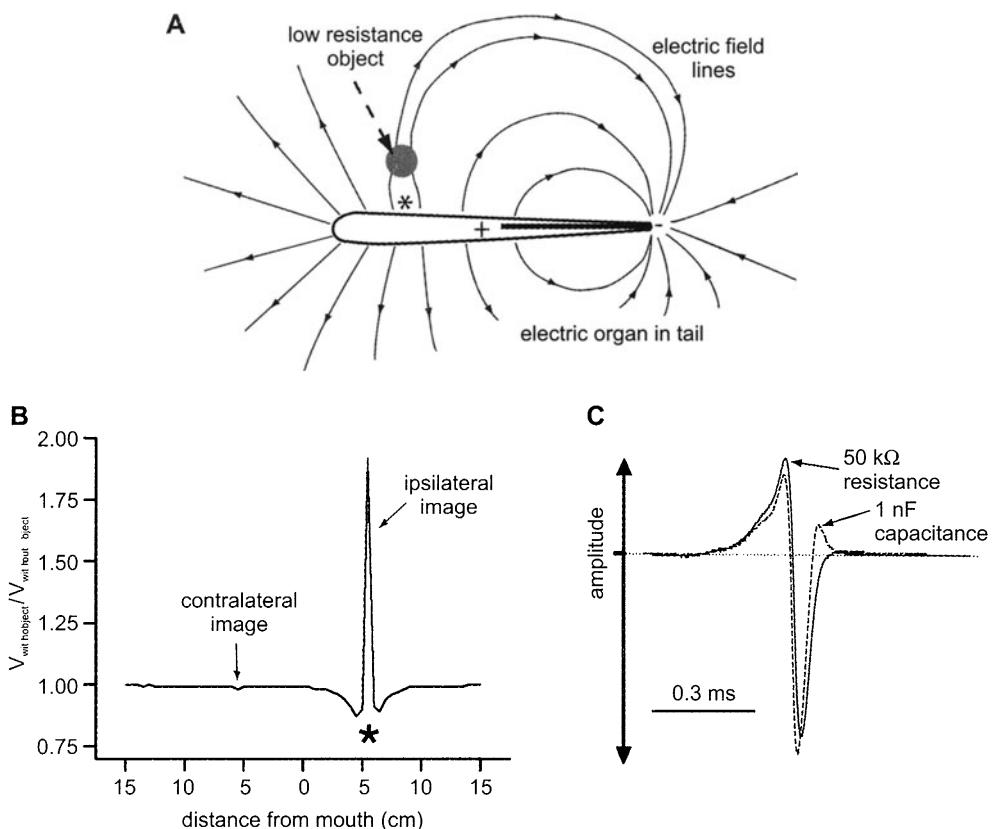
Fig. 7 Weakly electric fish generate an electric organ discharge by which they can locate and discriminate objects whose electrical properties differ from those of the water. An 'electric image', comprised of changes in the local transepidermal voltage, is formed along the body surface. (A) The EOD's field lines, along which current flows, converge towards a conductive object thereby increasing the local transepidermal voltage at the asterisk. Modified from Lissmann and Machin (1958). (B) The local EOD is 'modulated' strongly upwards at the point on the skin (*) nearest to the conductor and is modulated only very weakly downwards on the opposite side of the fish. 'Modulation' is defined as the ratio of the locally altered EOD voltage to the voltage measured with no object present. Modified from Caputi et al. (1998). (C) For a purely resistive object, the electric image changes amplitude over time with the waveform of the EOD. A capacitive object, however, can modify the time course of the local EOD-waveform as well. Two EODs recorded from *Gnathonemus petersii* in the presence of either a purely resistive or a capacitive object are shown normalized to their maximum voltages. An unmodulated EOD would overlie the 'resistive' EOD. Modified from von der Emde (1999).

μScm^{-1}. Once the *Daphnia* has been detected, the fish appears to electrically scan its prey while swimming backward. The *Daphnia* is moved forward relative to and alongside the fish's head to within a few millimeters of the mouth, where the fish makes a short lunge and sucks in the *Daphnia*. The whole process averages less than 700 ms from detection to ingestion with the fish continuously updating its estimate of the prey's position until the last few hundred milliseconds.

The *Daphnia* creates only a very minor perturbation of the fish's circa 1 mV local electric field. Nelson and MacIver (1999) estimate that a 2-3 mm *Daphnia* at 1 cm distance will produce about a 3.2 μV maximum perturbation on the skin surface with a half-maximum diameter of about 1 cm. This involves about 200 electroreceptors on the fish's well-endowed dorsal surface. Coupled with the relative prey to predator velocities, this suggests that the local electric field is modulated at about 4 to 5 Hz during detection and about 25 Hz during the rapid reversed swimming.

Structure of the Local EOD

The near-field EOD is a complex pattern over time and space that depends on the location and functional segmentation of the electric organ(s), electric organ synchrony and the non-homogeneous internal and skin impedances. The local EOD amplitude, field direction, d.c.-bias, phase and harmonics all vary along the length of a fish and through the course of an EOD cycle. The internal sources and sinks of EODs also move apart as the fish grows. For example, the highly synchronous firing of *Eigenmannia*'s electric organ results in a strongly dipolar EOD that alternates in polarity through each cycle (Fig. 2C). In contrast, the pulse-type electric organ of *Gymnotus carapo* is highly inhomogeneous and unsynchronized resulting in a more complex local field. The elongate *Gymnotus* body geometry and high tissue conductivity funnel the flow of nearby currents, of both internal and external origin, along the longitudinal axis to form an electrical 'fovea' near to the mouth where electroreceptor density is highest (Castelló et al. 2000). Within the fovea, the amplitude of the electric field is increased by up to several hundred percent and the field vectors are aligned perpendicular to the skin. Collimation of the electric field vectors presents the electroreceptors in the fovea with a maximally perpendicular stimulus, which is thus maximally effective and minimally spread. The anterior portion of the *Gymnotus* electric organ acts as a voltage source to supply localized current flow that is highly dependent on the nearby load. Just the thing for electrolocation. In the trunk and tail regions, the electric organ acts more as a current source that is independent of the external load. The local electric field is weaker and rotates through the EOD cycle, thus presenting a continuously changing image to a sparser array of electroreceptors. As a long dipole in a resistive narrow tail, the tail portion of the electric organ acts as a constant current source and serves a longer range of transmission than the more rostral electric organ segments. Thus the rostral EOD field appears to be specialized for electrolocation whereas the caudal field may be specialized for communication (Aguilera et al. 2001). Electric fields of mormyrids are relatively less complex because the electric organ is restricted to a small area of the tail peduncle.

The Electric Image

Weakly electric fishes can distinguish between capacitive and purely resistive impedances (von der Emde 1990, 1999). Purely resistive objects, such as rocks, alter the local EOD magnitude but not its shape. Despite the fact that a resistance will only change the local EOD magnitude, the electric image is not a purely multiplicative function of the local EOD amplitude (Fig. 7A). Rather, the perturbation is proportional to the component of the electric field directed towards

(or away from) the object ($E_\updownarrow$) and is thus largest where this field component is greatest (Rasnow 1996). A resistive sphere (either a conductor or an insulator) can be thought of as a dipole aligned parallel to $E_\updownarrow$ (Lissmann and Machin 1958, Bacher 1983). A conductor will draw the electric field lines towards it, locally increasing $E_\updownarrow$ and thus increasing the local current density to an extent that depends on the water resistivity (ρ_{water}).

$$\Delta J = \frac{\Delta E_\updownarrow}{\rho_{water}} \tag{11}$$

The transepidermal potential, the voltage difference that is sensed by the fish's electroreceptors, increases as the product of the local current density and the skin resistivity (ρ_{water}) and thus is a function of the relative resistivities of skin and water.

$$\Delta V_{skin} = \Delta J \rho_{skin} = \Delta E_\updownarrow \frac{\rho_{skin}}{\rho_{water}} \tag{12}$$

An insulator will have the opposite effect, bending current lines away from the object and lowering the transepidermal voltage but with half the magnitude (Rasnow 1996). Electric field perturbations are strongest when the electrical contrast between the object and the water is greatest and thus vary with water conductivity and EOD frequency. For example, at frequencies near to that of the EOD of Apteronotus, a single Daphnia produces a minimal impedance shift at water conductivities around 300 μScm^{-1} and is most easily detected at about 35 μScm^{-1} (MacIver et al. 2001).

The magnitude and sign of the electric image vary along the length of the fish. The image is maximal at the location along the fish from which $E_\updownarrow$ originates to intersect the object, usually closely adjacent to the object. The image is strongest on the side of the fish oriented toward the object and only very weak and inverted on the opposite side (Fig. 7B). The image center described above as an increase for conductors and decrease for insulating objects is surrounded by a small area of opposite sign (Scheich and Bullock 1974; Caputi et al. 1998).

The capacitance of biological tissue causes a change in the shape of the EOD (Fig. 7C). The form and extent of the distortion of the EOD-waveform (Budelli and Caputi 2000) depends upon the object's position and the time constants for charging and discharging of the object's capacitance and even on the water conductivity. For example, Rasnow (1996) estimates that a spherical leaf having the electrical characteristics of the water fern, *Hygrophilia* ($\rho = 200$ kΩcm and C = 76 $nFcm^{-2}$; Heiligenberg 1973) would shift the phase of an 800 Hz *Apteronotus* EOD by 3.5 to 38 degrees in water with resistivities of 5 to 50 kΩcm, respectively. The largest distortions likely occur at intermediate values of capacitance where a match occurs between the frequency content of the local EOD and the time constants ($=RC$) of the capacitance (von der Emde 1990). Spectrally broad, pulse-type EODs may supply a richer electrical image by simultaneously scanning a capacitance at multiple frequencies.

The effective range of electrolocation is roughly about the length of the fish. Even when the object is very close to the fish, the magnitude of the electric image is only a small fraction of the

local EOD measured with no object present (Rasnow 1996). As the object is placed at successively greater distances, the image magnitude diminishes exponentially with about the one-third power of distance. At the same time, the electric image also is blurred by spreading out along the fish. Gerhard von der Emde and colleagues (von der Emde et al. 1998) have shown that the mormyrid fish, Gnathonemus petersii, appears to use the ratio of image magnitude to image blur as a cue to the range of an object. The cue is only rarely unambiguous, however, as two objects that differ in shape may produce similar magnitude-to-spread ratios at different distances. When asked to choose the more distant of two such objects, the fish made errors at distances that could be predicted from the similarity of the magnitude-to-spread ratios.

The EOD-modulations that Apteronotus experiences when a Daphnia passes nearby are similar to those caused by a neighbor's EOD that is of similar frequency to its own EOD. The local EOD is modulated in amplitude and phase and at a rate of a few Hz to several tens of Hz. Apparently, the fish has difficulty distinguishing modulations caused by an object from those caused by a neighbor's EODs. Some species will shift their EOD frequency away from that of the jamming signal thus increasing the modulation rate caused by jamming and re-establishing a clear channel at lower modulation rates. The behavioral and neural basis for this 'jamming avoidance response,' thoroughly described by Walter Heiligenberg and his colleagues (Heiligenberg 1991), exemplify central nervous processing of electrosensory stimuli. The adaptive filter found in the ELL sets the stage for extraction of general stimulus properties relevant to the electric image. ELL neurons respond to stimulus timing and amplitude modulation. Midbrain neurons discriminate temporal modulations and/or differences across the body surface. The responses of still higher order neurons are more closely tied to behavior, pooling information on various parameters from across the body to discriminate stimulus features with higher acuity and greater specificity but with less sensitivity to irrelevant stimulus parameters.

COMMUNICATION

Passively produced bio-electric fields probably serve an important short-range communication function for most electroreceptive fishes but have received little experimental attention in this regard. Reproductive male stingrays are able to home to the deeply modulated bio-electric fields produced by females waiting buried in the sand (Tricas et al., 1995; Tricas and Sisneros, this volume). The extensive social body rubbing, mouth opening and gill flaring of elasmobranchs and catfishes probably involve extensive modulations of their bio-electric field that may be important display elements in their own right. Similarly, investigation of the communicatory function of the variable EODs of skates (Bratton and Ayers, 1987), and the Synodontid and Clariid catfish (Baron et al. 1994a; b) is only just begun. It is known, for example, that Synodontid EODs are modulated in temporal pattern, frequency and amplitude during social encounters (Baron et al. 1994a).

In contrast, an elaborate repertoire of electrical displays has been described for the weakly electric fishes. Individual EOD waveforms and, in the case of wave-type species, the repetition rate of EODs can identify the species, sex and reproductive and social status of a fish. Under hormonal control, EOD wave-shape and repetition rate may change developmentally,

seasonally and as a result of social interactions. Male *Eigenmannia*, for example, might engage in a night-long physical and electrical combat that results in the most dominant male having the lowest EOD frequency (Hagedorn 1986). Females also compete with the result that the dominant female has the highest frequency and receives the attentions of the dominant male. EODs may be useful for communication over distances of several meters depending upon the strength of the signal and the sensitivity of the receiver. Generally, the signals of larger fish are stronger and a favorite aggressive strategy for some species is to bite off a portion of the tail, and therefore the electric organ, of competing conspecifics.

The mormyrid knollenorgans are specialized for reception of the EODs of neighboring fish. Knollenorgans are more sensitive than the mormyromasts and, unlike the still more sensitive ampullary organs, are tuned to the peak frequencies of the EOD power spectrum. The knollenorgan pathway is specialized for speed and reliability of transmission. Knollenorgans fire a single spike with a fixed latency to a lumen-positive voltage transition. Activity is relayed over electrical synapses to cells within the ELL where responses to the animal's own EOD are eliminated by a brief inhibitory corollary discharge arising from the EOD command pathway.

Carl Hopkins and Andy Bass (1981) have demonstrated that these specializations of the knollenorgan pathway could allow male *Brienomyrus brachyistius* (*triphasic*) to distinguish the EODs of male and female conspecifics. Males emit EODs of 1.6 ms duration, but respond with courtship calls only to shorter duration stimuli, about 0.4 ms, that mimic the shorter EODs of females. A neighboring fish's EOD will be experienced at any point in time as inward flowing current on one side of the body and 180° out-of-phase (outward) current on the opposite side of the body. Through the course of an EOD, the polarities will reverse, but will remain in opposition for each successive phase of the EOD. Thus, in response to a female EOD, knollenorgans from one side of the body will experience a positive-going transition and fire spikes about 0.4 ms ahead of those on the opposite side of the body. In addition, knollenorgans having differing tuning curves respond best to different slopes of the positive going stimulus and may thus aid in discrimination of EODs from different individuals.

Modulation of the repetition rate of wave-type EODs and the pattern of intervals between successive pulse-type EODs form the basis for signals used in aggressive and courtship encounters. These signals take a variety of forms from simple accelerations or decelerations to brief phase-locking of EODs or EOD cessations and, of course, complex mixtures of a number of these (Hagedorn 1986; Hopkins 1986). EOD-interruptions ('chirps') of courting male *Eigenmannia* comprise a complete cessation of the EOD for up to several seconds, during which time a head-negative baseline is maintained. The resulting combination of low- and high-frequency spectral components elicits responses in both the ampullary and tuberous systems, respectively (Metzner and Heiligenberg 1991). Higher-level neurons combine both types of information to respond selectively to chirp-like stimuli (Heiligenberg et al. 1991).

The fish might distinguish self-generated EOD-interruptions from those of its neighbors by comparing the pattern or timing of ampullary responses over its body surface. A chirping fish will experience the same head-negative baseline over its entire body surface whereas a neighbor

will feel opposite polarity offsets to either side. The basal firing rate of ampullary receptors shows an increase-decrease pattern in response to negative-offset EOD-interruptions and a reversed pattern for positive-offsets (Metzner and Heiligenberg 1991).

The recent work from Angel Caputi, Omar Trujillo-Cenóz and others (Aguilera et al. 2001) suggests an as yet unexplored means of communication between weakly electric fish. The electric fields generated by neighboring fish vary along the length of the receiving fish in relation to the neighbor's size, orientation and distance. One can imagine a vast array of social interactions based merely on the advantageous presentation of one's electrical 'body image.'

Acknowledgements

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16

Essay: The Lure of Field Research on Electric Fish[1]

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Since 1979, I have spent many years studying electric fish in wild and remote jungle rivers of Central America, Africa, and the Amazon. It is one of the most fascinating and biologically rich yet dangerous occupations in the world. There is no 24-hour emergency medical care. A snake bite or fall from a tree could be lethal. Regardless, I rarely thought of these negative aspects of my work. Recently, after the death of a close friend who drowned while collecting fish, I began questioning my motives in pursuing such a dangerous lifestyle. I wondered what drove me and my colleagues who work on electric fish to do fieldwork.

I spoke with a variety of scientists who have undertaken electric fish field studies over the years. Although they told me a great deal about their research, they didn't seriously address why they went into the field. I found this question very difficult to answer for myself as well. In the end, I think it is a sense of love-—a love of being alone with animals in nature and knowing that their natural behavior may hold the answers to so many important biological mysteries. While formulating his ideas on speciation, Darwin spent hours watching ants in his backyard, glued to the ground. His love of nature and far-flung and tireless fieldwork made him ask one of the most profound questions in our history: "Is life static, or does it change over time?" In textbooks, we often read the results of fieldwork in an objective, scientific format, but we rarely glimpse the thoughts and emotions that motivate the scientific process. This essay is a brief view of this process.

Dr. Theodore Bullock of the Scripps Institution of Oceanography is a role model for students and colleagues pursuing answers to questions about neurobiology and comparative biology around the globe. He was also one of the original driving forces in electric fish research. He created a lab policy to encourage physiological experiments that were best solved in the

Key words: Field work, electric fish, Amazon

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[1]In memory of Fonchii Chang

field. When I asked Ted what inspired him, he credited Dr. Per F. "Pete" Scholander, Head of the Physiological Research Laboratory at Scripps (1963-1970). Pete went into the field to perform physiological experiments that could not be done in the lab; he tested why the blood of cold water fish doesn't freeze and how sap rises in trees against gravity. We've all read about these studies in textbooks, but Pete's fieldwork sparked them. In (1966), he convinced the National Science Foundation to fund a floating laboratory called the Alpha Helix for just such indispensable physiological field inquiries in marine and freshwater ecosystems. A group of about ten scientists boarded the Alpha Helix in 1967 for the Amazon to study the ecology and physiology of South American gymnotiform fish, which Hans Lissmann in the 1950s had discovered produced weak electric signals. This trip and the interaction of many of these scientists engaged the attention and imagination of students, postdocs, and colleagues, and encouraged them to go into the field and see for themselves. Shortly after the Alpha Helix's voyage, some of the most lengthy and intimate studies of electric fish were done by Dr. Carl Hopkins in the rivers on the Guyanan shield and Dr. Horst Schwassmann in the Amazonian rivers.

I and many of my colleagues have worked throughout Central and South America and Africa. We did not necessarily set out to become field biologists; it seemed to happen serendipitously. Perhaps an advisor suggested going into the field, or experiments in the lab were not going as well as planned, or someone needed to collect a certain species, or needed a change of scenery. Most reasons for going into the field were first human and then accommodated the passion for science. Without a doubt, the major reason that people go into or remain in the field is their love of nature. We are born with a love of nature, especially toward animals. We can see it clearly in the faces of enthralled children as they watch a whale swim gracefully and silently by in a tank at an aquarium or a prairie dog bobbing in its burrow at a zoo. Many field biologists seem to maintain this child-like love of nature.

What inspires most field biologist is a sense of adventure and wonder. Each day brings something new, absolutely surprising, and completely absorbing. A colleague related this story: "One evening, I was watching an idyllic scene by a shallow, wide stream flowing past a stand of trees with some bitterns and herons in the Llanos. I saw an iguana come out of the bushes and leisurely wend its way down to the water to drink. Suddenly, the calm was broken by a huge splash as an alligator appeared at the shore, gobbled up the iguana, and disappeared."

When you are alone in a rainforest, you feel as if you are the only person on earth. It is an extraordinarily magical feeling to experience fully through all your senses. I lived in a tent in a very remote part of the Amazon studying the biodiversity of electric fish in Parqué Manu in Peru. Every morning at around 4 A.M., the frogs would end their chirping, while the howler monkeys began grunting and calling in the trees above, followed by a chorus of birds, the toucans and macaws making their "gritching", saw-like song, and finally the oropendolas gurgling and laughing as dawn approached. It was the most wonderfully *soothing* cacophony - nothing like the irritating urban ruckus of horns and garbage collection.

The rainforests are Edens with amazing mysteries unfolding before your eyes. Although every time I return to the rainforest, I experience intense pleasure at familiar sights, sounds, and smells, it is also a great joy to watch people experiencing it for the first time and watch them

light up with wonder. One colleague described some of these moments: "stepping into a stream, turning on your amplifier, and hearing the water come alive with the squeals and buzzes of the electric fish you have been reading about for years" or "watching a pair of bat falcons sit above your stream every night at dusk, picking off bats as they fly from the forest." Another felt that standing in a forest stream, he was "transported . . . back to the time of the great naturalists, such as Darwin, Russell or von Humboldt." He felt a sense of "adventure and accomplishment when experiments that seemed so rickety, because they were held together by string, tacky wax, and duct tape, turned out successfully."

Many field biologists are patient and willing to watch and collect data for hours on end, because they want to know how things work in a natural setting. For me, animal behavior informed all aspects of neurobiology and preceded any reductionistic laboratory investigations. For example, I wanted to understand many of the overt behaviors and social signals surrounding breeding behavior of electric fish, before I asked how the fish produce these signals or the larger question: how does one small aspect of the fish brain and nervous system work?

When I began these studies, the jamming avoidance response of electric fish was being used to probe the sensory input and motor output of the fish's nervous system. Moving from cell to cell within the brain's hierarchy, we could ask the cell, do you process this behavioral information and, if so, how? Because of its utility in identifying the electrical processing circuitry of the fish's brain, the jamming avoidance response was a very important and well-described behavior. During the behavior, one fish shifts its electric organ discharge frequency away from a nearby neighbor's similar frequency to avoid being jammed. And as well described as this behavior was in the laboratory, no one had ever recorded jamming events from fish in the field. Why develop a whole behavior, and not use it? Why not just move out of range of the other fish? There had to be some other context for this ability.

There was, and it was found by field and laboratory breeding observations. In some species, breeding is determined by dominance, which is denoted by the fish's electrical frequency. Imagine if our tone of voice determined whether we could breed or not; we would learn to modulate it pretty quickly! Non-dominant fish will fight physically with the dominant fish. The winner will maintain or shift their signal to the dominant frequencies, thus ensuring their right to breed. This amazing ability to detect and to change their electric frequencies is critical to the species' breeding process, and encompasses the jamming avoidance response.

The sensory-motor behavior of electric fish is one of the best-described neural systems in neurobiology today, a *tour de force* of collaborations between behaviorists, anatomists, and neurophysiologists. However, my colleagues felt it is important not to underestimate the social complexity of so-called simple organisms and the role that signaling, electric or otherwise, plays in the social system. We believe that our field observations changed our understanding of electric fish biology. Generally, our work yielded broader insights into how electric fish use their electric organ discharges in their natural environment during courtship, agnostic encounters, electrolocation, and feeding. These observations led to a series of laboratory experiments to determine how electric fish can physiologically produce some of the signals observed in the field. Ultimately, new neural pathways and physiology were discovered based on these field behavioral observations.

The answers from field observations can be very satisfying, because they fill in the holes in our understanding of a subject and pique new questions. However, the obstacles to mounting a field expedition are monumental. Funding, family obligations, fear, health, governmental disputes, military intervention, advisor's lack of interest, work obligations, weather, and the possibility of being hurt or killed are all problems that may intervene and must be solved before or during the circumscribed period of the study. Almost everyone gets sick in the field, from malaria to *E. coli* poisoning to a variety of parasitic diseases. Darwin suffered his whole life from an illness he contracted during his voyage on the Beagle, most likely while he was in the Amazon. Vulnerability is part of life in a jungle.

Many of my colleagues briefly described some of the obstacles they faced in going to, or while in, the field. However, my initial field expeditions in the Amazon encompass many of my colleagues problems, so I will relate the obstacles as a single story. I was a complete neophyte concerning jungle exploration when I set off for an area near the Colombian, Venezuelan, and Brazilian border called San Carlos de Rio Negro. Many famous jungle explorers, such as Spruce, Humboldt, and Bates, had traveled in this area. I had been invited by a colleague, Kate Clark, to join her at a research station that she was running with her husband. Kate had spent three years collecting fish in this area, and my mission was to find out what species of electric fish lived in the area and how easy it would be to carry out a research project there. Some important logistical questions were: do they have a reliable source of electricity at the station? Did they have boats with functional outboard motors? Could you buy gasoline and oil for outboard motors? What equipment was available at the research station; for example, scales, alcohol, formaldehyde, and jars for weighing, preserving, and storing fish?

I left for Caracas, Venezuela, with what I considered a substantial amount of money—$60. I was a graduate student, making $600 a month, $400 of which went for tuition and rent; $60 was a tenth of my monthly income. I landed at the International Airport, where the customs officials were extremely jovial and polite. However, my first evening in Caracas, I learned that the cheapest hotels were $60, and unbeknownst to me, my credit card had just expired that week. Fortunately, Dr. Mago-Leccia, a colleague of my advisor, Dr. Walter Heiligenberg, lent me money for supplies, gave me letters of recommendation and collaboration, and dropped me off at the National Airport, so I could fly down to San Carlos.

I was headed toward the plane, when an airport official checked my documents. She noticed that those jovial customs officials had not stamped my passport. I was in the country illegally and would have to wait several more days to straighten it out. As I was staring at her with complete idiocy, the travel agent who had checked me in wandered by. The official immediately launched an even louder tirade against him for not noticing my illegal status, whereupon a whole group of people gathered and joined in the argument. Finally, I had the presence of mind to pull out the carefully written and rather imposing documents that Dr. Leccia had given me. All conversation stopped as they read them carefully. Then they waved me through the checkpoint, exclaiming, "Why didn't you say this before?" I felt like Dorothy, supplicating the guard at the entry to Oz— this trip was definitely not going to be easy.

In the airplane, I worried about when my illegal status would next be noticed. I landed in Puerto Ayacucho, the capital of the Amazon region in Venezuela and told the airlines that I wanted to go on to San Carlos. They shook their heads slowly as they gazed at the thunderclouds massing in the sky. Maybe tomorrow. Meanwhile, they shuttled me into downtown Puerto Ayacucho, where they dropped me at the Mirabel Hotel. I could get a room here for $5 a night, but I would have to be happy with no running water and rats running in the rafters overhead. This tradeoff seemed reasonable, considering my financial status. I had three weeks of fieldwork ahead of me, and I still had to buy my food.

The first night in the hotel was the high point of the trip, because a fellow traveler invited me to a fiesta. It was a lovely warm night, and young and old people were dancing romantically to Latino waltz music in a Hemingway-like bar along the river under the moon. Three nights later, still waiting in Puerto Ayacucho for a plane, my supply of money was quickly diminishing. With what remained, I bought some onions, beans, and rice and hoped that I could live on this diet for three weeks. That night the weather improved, and I was told to be ready at 5 A.M. to fly to San Carlos. I was extremely excited and spent the evening reading my Spanish language texts in the central courtyard of the hotel. I was getting ready to return to my stifling cubicle, when a very intense man sat down next to me. He asked me what I was doing, and with my limited Spanish, I tried to explain my project. He sat there for a moment and then said that he would like to make a present of himself to me. Suddenly, the courtyard seemed very empty, and I feigned complete ignorance while I gathered my books. He grabbed at my arm, and in the best and most brutal Spanish that I knew, I told him not to bother me now and ran to my room, piling all the furniture against the door. Minutes later, the electricity went out. Dawn seemed to come days later, and I was exhausted from a night of high adrenaline, making sure the furniture did not move.

At the airport, I climbed into the front seat of a six-passenger plane with two other people. We soared over the Amazonian jungle. Green rolled in every direction for miles. After twenty minutes, the pilot seemed restless and started reading the newspaper. I felt a little uneasy, as I could see the trees pretty clearly, so we were not very high in the air. I didn't think that things could be much worse than a night spent in fear of a mad macho, but after ten minutes of perusing the news, the pilot put the paper over his head and promptly fell asleep. I looked around at my fellow passengers, who seemed completely nonplussed by this occurrence. I busied my self with my camera and took three roles of film in the twenty minutes that he napped. When we finally landed on the dirt runway in San Carlos, I felt like kissing the ground, but I knew that I still had one more hurdle - the airport guard. He opened my passport and examined it painfully slowly, page by page, and finally handed it back to me. I was so frightened of being sent back that I grabbed the passport and ran down the road toward the field station. I burst in on Kate Clark and rattled off all the traumas I had experienced in a mere five days, and she laughed and said, "Don't worry - the guard at the airport can't read; he was just looking at the pictures!"

Kate showed me around the field station. As I unpacked my meager food stores, she graciously said that we all shared food. I knew she thought my offering was rather pathetic, but she never let on. We had many wonderful meals together, and she told me a great deal about the

social structure of San Carlos. It was the site of a military post, because it was on the border with Colombia, but, in addition, many Yanomami Indians from the interior had settled in town. The Yanomami suffered greatly as they tried to adjust to Western civilization. She recounted a very sad story about one of her assistants who had asked her husband to buy him a pair of boots when he went back to the States. Kate's husband asked him why he needed boots in such a warm place as San Carlos, and the assistant replied, "If I have a pair of boots, then I can be a man like the others" meaning the military men.

The water in the Rio Negro was like black tea. We bathed in the clear black water with schools of gold-sided piranha swimming close by. Kate had fished in a number of areas, small inlets, big rivers, and lakes. One afternoon, she and her husband were snorkeling in a small inlet, when she noticed something large swimming underneath her. As she tried to maintain a calm stance on the surface, a thirty-foot anaconda swam below her. I knew that I was in capable hands, since she was able to remain motionless on the surface, while in proximity to one of the largest predatory snakes in the world.

Kate had an excellent fish collection, which she proudly showed me while I photographed the electric fish species. One evening, we loaded our gear into a dugout canoe and headed upstream toward some rapids in the Casiquiare Canal, approximately ten miles away. A full moon rose early over the river just as the sky turned a dark velvet blue. Frogs were chorusing as we started collecting electric fish with our seine in the rapids. After an hour of work, finally someone cried out, "Hey! Where's the boat?" In our haste to get into the water, the fishermen had not secured the boat, and it was laboriously circling in a small whirlpool in the middle of the river, or so everyone said. I had left my glasses in the boat, because they had fogged up. Unfortunately, I was the only one who could swim, but I couldn't even see the boat! I was going to have to tow someone with me to guide me to it. The group decided that was not a great plan, but we also knew that it was not safe to stay by the river at night without any sort of shelter, because in a large rainstorm, we would quickly lose body heat and become hypothermic. We could see a light on the far shore in a bar in Columbia and tried to attract attention by whistling and screaming. After thirty minutes of this, we were hoarse and needed a new plan. We decided to build a fire. We scoured the banks for driftwood, but it was too wet. The only dry fuel available was my field notebook. It burned brightly and attracted some passing fishermen, who brought us to our boat and ultimately back to the station. As I snuggled down into my hammock that night, warm and dry, a fierce rainstorm blew up and battered the field station, and I swore I would never return to San Carlos again. As fate would have it, I was there one year later.

Many unexpected events in the field can change or even endanger your life. During 1984, I was working in Georgetown, The Gambia, studying the reproductive behavior of riparian electric fish populations. I was particularly interested in the breeding and nesting behavior of *Gymnotus niloticus*, a large electric fish about the size of a dolphin, ranging from five to seven feet. Georgetown was a very poor fishing community at the time, with no hotels, so I rented a room from a family. Most electric fish breed during the rainy season, but my study took place during the beginning of the Sahel drought. The rains never came that year, and the fish did not breed in the river. I was very disappointed at what seemed to be the failure of my field season,

but at the suggestion of Carl Hopkins and Hans Lissmann, I collected and examined Synodontid catfish. These turned out to be a whole new class of electric fish, the weakly electric catfish. The excitement of this discovery balanced the failed breeding season.

The most unexpected event for me, however, was how profoundly the African culture changed by life. The Gambia was one of the poorest countries on earth, yet the warmth, friendship, and sharing that I experienced was unparalleled. I have never experienced a community so connected to one another that social programs seemed unnecessary. The elderly and hungry are cared for by every person, and the children are protected by all adults. It was one of the richest human experiences I have ever had.

For the last ten years, I had the good fortune to work with two excellent ichthyologists in Peru, Dr. Hernan Ortega and Fonchii Chang. During the years of the Shinning Path's terrorist activities and the subsequent destabilization of the Peruvian economy, scientists at governmental institutions received minimal or no pay. Regardless, both of my colleagues continued to go into the field describing the Peruvian ichtyofauna. I have so much admiration for their strength of conviction, because given the same circumstances, I know I would have given up. Fonchii mounted several expeditions in Peru, often working only with a student or a local fisherman. On one occasion in southern Peru, she was seining with a student, and he apparently stepped on an electric eel. He was either killed outright or stunned; he fell into the water and was swept away. Fonchii worked alone for hours searching for her colleague. The hardest thing she had to do was to tell his parents that their brilliant son was dead. Sadly, Fonchii was drowned three years later in an accident while trawling for deepwater specimens on the Rio Pastaza.

These experiences were a part of my life as a scientist for which graduate school had not prepared me. When we step out of the controlled environment of the laboratory, we step into the chaotic events of life that surround our field endeavors. It is fascinating, humorous, surprising, bug-bitten, frustrating, beautiful, physically demanding, emotionally and intellectually gratifying, and sometimes wrenchingly sad. But it is life intersecting science in a synergistic way by broadening our perspective on political, social, economic, and religious beliefs, and ultimately enriching and humanizing our science.

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